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GENERAL METHODS FOR THE ANALYSIS OF FOLPET IN OILY AND NON-OILY CROPS: ENFORCEMENT METHODS

(VERSION 1.0 FOR APPLES, AVOCADOS, CANTALOUPE, CRANBERRIES, CUCUMBERS, GRAPES, LETTUCE, ONIONS, STRAWBERRIES, AND TOMATOES)

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No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B) or (C).

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I. INTRODUCTION AND SUMMARY

A. Scope

Folpet is a protective fungicide produced by Makhteshim Chemical Works. The active ingredient, N-[(trichloromethyl)thio]phthalimide, controls certain noxious fungi in/on fruits, berries, vegetables, flowers, and ornamentals. Residues resulting from the application of folpet are regulated by the United States Environmental Protection Agency. Hence, a method is required for analysis of folpet in these commodities.

This document describes analytical techniques and procedures for analysis of folpet residues in a variety of commercially important crops. Information contained in this general method was derived from four source documents: (i) the original Analyst Ltd. method #FP/15/93 for analysis of folpet in non-oily crops[1], (ii) the original PTRL West method #568-W for analysis of folpet in oily crops[2], (iii) an independent laboratory validation at Horizon Laboratories, Inc. (HORIZON) of both original methods[3], and (iv) a letter[4] from A. J.

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[1]"A Method for the Determination of Folpan and Phthalimide Residues in Non-Oily Crops", Method FP/15/93, by H. M. Schlesinger, Analyst Ltd., Kiryat Weisman, Rehovot, Israel, March 4, 1992.

[2]"A Method for the Determination of Folpet Residues in Avocados and Other Oily Crops", Method 568-W, by L. T. Nishioka, *et. al.*, PTRL West, Inc., 4123-B Lakeside Drive, Richmond, CA, March 5, 1996.

[3]"Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", Study #10146, by M. Williams, Horizon Laboratories, Inc., 1610 Business Loop 70 West, Columbia, MO, June 4, 1996.

[4]"Validation of Folpet in: Avocados, Tomatoes, Lettuce, and Onions", a letter from A. J. Krynitsky (Chemist, EPA) to E. Zager, (Acting Chief, Chemistry Branch I/Tolerance Support, Health Effects Division, EPA), September 30, 1996, based upon the US EPA Analytical Chemistry Laboratory's Petition Method Validation (PMV).

Krynitsky (EPA) to E. Zager (EPA), dated September 30, 1996.

B. Principle

For non-oily crops[5], folpet is extracted from freshly macerated sample with acidified ethylacetate (EtOAc)/sodium sulfate. Solids are filtered from the extract and discarded; the extract is diluted to a known volume with EtOAc. An aliquot is partitioned against aqueous phosphoric acid, which is discarded after phase separation. The EtOAc extract is desiccated through anhydrous sodium sulfate, vacuum-evaporated to dryness, then dissolved in hexane. Further purification is effected by Florisil® column chromatography and, for onion matrix, C-18 reverse-phase column chromatography. Quantification of folpet in the final extract is performed by gas chromatography with electron capture detection (GC/ECD). For non-oily crops, this procedure has approximate detection and quantification limits for folpet of 0.01 ppm and 0.05 ppm, respectively.

For oily crops[6], folpet is extracted thrice from freshly macerated sample with acidified EtOAc/sodium sulfate. Solids are filtered from the extract and discarded, and the extract is diluted to a known volume with EtOAc. An aliquot is vacuum-evaporated to just-dryness, then the residues are dissolved in acetonitrile (ACN). The ACN extract is partitioned against hexane; after separation, both phases are retained to this point. The ACN phase is back-partitioned twice against hexane. All hexane phases are pooled, then partitioned twice against ACN. The ACN phases are pooled and vacuum-evaporated to just-dryness; the hexane layers are discarded as waste. Dry residues from the ACN phase are dissolved in methylene chloride (DCM):acetone. Further purification is effected by gel-permeation chromatography and, if appropriate, Florisil® and C-18 reverse phase column chromatography. Quantification of folpet in the final extract is performed by GC/ECD. For oily crops, this procedure has approximate detection and quantification limits for folpet of 0.01 ppm and 0.05 ppm, respectively.

Figure 1 presents the chemical structure of folpet.

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[5]E.g., apples, cantaloupe, cranberries, cucumbers, grapes, lettuce, onions, strawberries, and tomatoes.

[6]E.g., avocados.

II. MATERIALS AND METHODS

A. Equipment

Equipment	Suggested Manufacturers:[7]
Acrodisc Filter, CR PTFE 0.45 μm, 13 mm dia.	Gelman
Analytical Balance	Ohaus GA110
C-18RP Cartridges (Optional) Mega Bond-Elute, 1 gram, part #1225-6001	Varian, Harbor City, CA
Filter Paper, Whatman #1	Fisher Scientific
Florisil® Cartridges (Optional) Bond-Elute, 1 gram, part #1211-3049	Varian, Harbor City, CA.
Food Processor with glass jar and maceration blade	Local Supply
Gas Chromatograph with Electron Capture Detector Mega-bore Capabilities Split-Splitless Injector	Hewlett-Packard Model 5890 Series II
Gas Chromatograph Column: DB-1	J. and W Scientific, 30 M X 0.53 mm i.d., 1.5 μm film thickness, Cat. #125-1032
Gel-Permeation Chromatograph	ABC Instruments, Columbia, MO
Column: 600 mm x 25 mm dia., glass, packed with 40 grams of BioRad BioBeads S-X3 (200-400 mesh) swelled overnight in DCM:acetone (3:7, v:v).	
General Laboratory Glassware	Various
Glass Columns: 11 mm i.d. X 25 cm, equipped with a teflon stopcock and a 200 mL reservoir	Fisher Scientific, #K420280-0213

[7]Equivalent sources of the listed equipment and reagents may be used.

Glass Wool	Fisher Scientific
Ice, Wet	Local Supply
Vacuum-Evaporator*	Fisher Scientific
Single Pan Balance	Ohaus E400
Sonicator	VWR Scientific
SPE Column Manifold with vacuum source	J. T. Baker
Weighboats, Plastic	Fisher Scientific

B. Reagents and Standards

Acetone: Fisher Optima Grade

Acetonitrile (ACN): Fisher Optima Grade

40% ACN:Water: Dilute 400 mL of ACN to 1 L with water. Mix and store at ambient temperature.

60% ACN:Water: Dilute 600 mL of ACN to 1 L with water. Mix and store at ambient temperature.

Di(ethyleneglycol)diethylether: Sigma/Aldrich
(i.e., DEGDEE), HPLC grade

2% DEGDEE in Hexane: Dilute 20 mL of DEGDEE to 1 L with hexane. Mix and store at ambient temperature.

Diethylether (EtOEt): Fisher Anhydrous
Ethylacetate (EtOAc): Fisher Optima Grade

Florisil®: 60-100 mesh, Fisher Scientific

Activate Florisil® overnight at 150°C. Cool and store in a desiccator; the reagent is stable for 12 months.

Hexane: Fisher Optima Grade
Methanol: Fisher Optima Grade

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*Vacuum-evaporator = rotary evaporator.

1.0% Methanol in DCM: Dilute 10 mL of methanol to 1 L with DCM. Mix and store at ambient temperature.

Methylene Chloride (DCM): Fisher Optima Grade

DCM:Acetone (3:7, v:v): Mix 600 mL of DCM with 1.4L of acetone. Mix and store at ambient temperature.

Phosphoric Acid (85%): Fisher Scientific (ACS grade)

0.75% Phosphoric Acid: Carefully, add ca. 8.8 mL of 85% phosphoric acid to 1 L of water. Mix and store at ambient temperature.

Sodium Sulfate (anhydrous): Fisher Scientific (ACS grade)

Water: Distilled and/or deionized
Folpet analytical standard, available from MANA

C. Analytical Procedure: Non-Oily Crops

C.1 Sample Preparation: Non-Oily Crops

Non-oily sample matrices analyzed for folpet must be stored frozen, un-ground and intact. Folpet is not stable in macerated tissue, even during frozen storage. Hence, matrix is macerated and subsampled just prior to analysis.

Place ca. 500 g of partially-thawed or frozen sample into an appropriate food processor, then chop/macerate to homogeneity. Retain a representative 25 gram aliquot for **immediate** extraction. Discard the remaining homogenized sample (Note 1, p. 24).

C.2 Extraction: Non-Oily Crops

a. **Immediately** transfer the 25 gram sample into a glass blender jar. Quality control fortifications into untreated control (UTC) matrix are made at this point (see Section F.1).

b. **Immediately** add 100 grams of anhydrous sodium sulfate, 220 mL of EtOAc, and 2 mL of 85% phosphoric acid to the sample (Note 2, p. 24). Blend at medium speed for 2 minutes.

c. Vacuum-filter the sample through Whatman #1 filter paper (seated in a Büchner funnel) into a 250 or 500 mL graduated cylinder (Note 3, p. 24). Rinse the blender jar and solids with 30-40 mL of EtOAc, then filter. Discard the filtered solids; adjust the extract volume to 250 mL with EtOAc, then mix.

C.3 Partitioning: Non-Oily Crops

a. Transfer 50 mL of the filtered extract into a 125-mL separatory funnel and add 10 mL of 0.75% aqueous phosphoric acid.

b. Mix the phases vigorously (ca. 1 minute). Allow the phases to separate (Note 4, p. 24). Discard the aqueous phase.

c. Place a plug of glass wool into the neck of a conical, glass funnel. Add 25-50 grams of anhydrous sodium sulfate. Pre-wash the sodium sulfate with 25 mL of EtOAc (Note 5, p. 24); discard this wash.

d. Percolate the lower, organic phase from C.3.b through the sodium sulfate into a 250 mL evaporation flask. Rinse the sodium sulfate with 25 mL of EtOAc; pool the rinse with the original extract. Vacuum-evaporate (<35°C) the sample to just-dryness.

C.4 Florisil® Chromatography: Non-Oily Crops

a. Prepare a Florisil® clean-up column as follows: Seat a small plug of glass wool into an 11 mm i.d. glass column. Rinse the column and glass wool with EtOEt and allow to air dry. Add 1 gram of fully-activated Florisil® to the column. Pre-wet the Florisil® with 1 mL of hexane and discard the eluant, if any. The wetted column is now ready for use.

b. Dissolve the dry residue from C.3.d in 5 mL of hexane. Transfer 2 mL of the solution to the top of the Florisil® column, taking care not to disturb the column surface. Gravity-perco-

late the sample into the column (ca. 2 drops/second), then wash the column sides with 6 mL of hexane. Percolate the rinse through the column. Discard all eluants to this point (Note 6, p. 24).

c. Elute folpet from the column with 5 mL of DCM containing 1% methanol (Note 7, p. 24) into a 25 mL evaporation flask.

d. Vacuum-evaporate (<35°C) the eluant to just-dryness. Dissolve the dry residue in a known volume of 2% di(ethyleneglycol)diethylether (DEGDEE) in hexane (for LOQ residues, generally 4 mL). Dilute with the same solvent as necessary to maintain the analyte concentration within the standard curve range. Submit the sample for GC/ECD analysis as described in Step E.

C.5 C-18RP Chromatography: Onions (Optional)[9]

a. Beginning with the extract from C.4.c, vacuum-evaporate (<35°C) the eluant to just-dryness. Dissolve the residue in 2 mL of ACN (with sonication and swirling), then add 3 mL of water and mix (Note 8, p. 25).

b. Prepare a C-18RP SPE cartridge as follows: Place the cartridge onto a commercial manifold vacuum system. Wash the column with 2 x 5 mL of ACN, then 2 x 5 mL of water, by pulling a vacuum sufficient to elute the column at ca. 5 mL/minute. **Do not let the column go dry at any point in this procedure.** The column is now ready for use.

c. Percolate the entire sample from C.5.a through the column with vacuum (1 → 2 mL/minute). Wash the column with 15 mL of 40% ACN:water (v:v); discard all eluants to this point. **Do not let the column go dry at any point in this procedure.**

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[9]C-18RP column clean-up of onion extracts was developed by HORIZON during the independent laboratory validation study[3]; the as-written Analyst Ltd. method failed to clean-up the extracts in a acceptable manner. C-18RP clean-up was also required during EPA's validation research for LOQ fortifications in onions[4].

d. Elute folpet from the column with 15 mL of 60% ACN:water (v:v) into a 50 mL evaporation flask (Note 9, p. 25).

e. Vacuum-evaporate (<35°C) the eluant to dryness. Dissolve the dry residue in a known volume of 2% DEGDEE in hexane (for LOQ residues, generally 4 mL). Dilute with the same solvent as necessary to maintain the analyte concentration within the standard curve range. Submit the sample for GC/ECD analysis as described in Step E.

D. Analytical Procedure: Oily Crops

D.1 Sample Preparation: Oily Crops

Oily sample matrices analyzed for folpet must be stored frozen, un-ground and intact. Folpet is not stable in macerated tissue, even during frozen storage. Hence, matrix is macerated and subsampled just prior to analysis.

Place ca. 500 g of thawed or frozen sample into an appropriate food processor, then chop/macerate to homogeneity. Retain a representative 25 gram aliquot for **immediate** extraction. Discard the remaining homogenized sample (Note 1, p. 24).

D.2 Extraction: Oily Crops

a. **Immediately** transfer the 25 gram sample into a glass blender jar. Quality control fortifications into UTC matrix are made at this point (see Section F.1).

b. **Immediately** add 100 grams of anhydrous sodium sulfate, 150 mL of EtOAc, and 2 mL of 85% phosphoric acid to the sample (Note 2, p. 24). Blend at medium speed for 2 minutes.

c. Carefully decant the extract from solids in the blender through a bed of pre-washed (25 mL EtOAc) anhydrous sodium sulfate (20→25 g) contained in a conical, glass funnel plugged with glass wool (Note 5, p. 24). Re-extract the solids retained in the blender with 2 x 50 mL of EtOAc; decant and filter the extract each time. After the final extraction, wash the funnel contents (now containing all solids from the last extraction) with 25 mL of EtOAc,

then pool all extracts in a 250 or 500 mL graduated cylinder (Note 3, p. 24). Dilute the entire sample with EtOAc to 250 mL, then mix. Discard all solids.

D.3 Partitioning: Oily Crops

a. Transfer 50 mL of the filtered extract into a 250-mL evaporation flask and vacuum-evaporate (<35°C) to just-dryness.

b. Add 25 mL of ACN, 100 mL of hexane, and ca. 1 gram of anhydrous sodium sulfate (Note 10, p. 25). Swirl the sample to dissolve the residue.

c. Transfer the solution (but not the sodium sulfate) to a 250-mL separatory funnel, cap, and mix the phases vigorously (ca. 1 minute). Allow the phases to separate (Note 4, p. 24). Retain both phases.

d. Partition the ACN phase from D.3.c against 2 x 50 mL of hexane. Retain all phases. Pool the hexane phases in a 500-mL separatory funnel and back-wash with 2 x 25 mL of ACN. After phase separation, pool all ACN phases (containing the folpet analyte) and vacuum-evaporate the sample to just-dryness (<35°C). Discard the hexane phase.

D.4 Gel-Permeation Chromatography: Oily Crops

a. Dissolve the dry residue from D.3.d in 7.5 mL of DCM:acetone (3:7, v:v). Filter the sample through a 0.45 μ Acrodisc filter to remove particulates.

b. Inject the sample onto a gel permeation chromatograph (GPC) housing a 40 g BioRad BioBeads S-X3 (200-400 mesh, size-exclusion gel) column equilibrated with the 3:7 DCM:acetone solvent (Note 11, p. 25).

c. Elute the column with the 3:7 DCM:acetone solvent with the following suggested operating conditions:

Column flow: 5 mL/minute

Dump time:	10 minutes (50 mL)
Collect time:	10 minutes (50 mL)
Wash time:	10 minutes (50 mL)

Optional GPC operating conditions suggested by the EPA[4] are:

Column flow:	5 mL/minute
Dump time:	19 minutes (95 mL)
Collect time:	5 minutes (25 mL)[10]
Wash time:	5 minutes (25 mL)

d. Vacuum-evaporate (<35°C) the "collect" fraction to just-dryness. Dissolve the dry residue in a known volume of 2% DEGDEE in hexane (for LOQ residues, generally 6 mL). Dilute with the same solvent as necessary to maintain the analyte concentration within the standard curve range. Submit the sample for GC/ECD analysis as described in Step E.

If GPC affords unsatisfactory sample clean-up, proceed to step D.5 below.

D.5 Florisil® Chromatography: Oily Crops (Optional)[11]

a. Prepare a Florisil® clean-up column as follows: Seat a small plug of glass wool into an 11 mm i.d. glass column. Rinse the column and glass wool with EtOEt and allow to air dry. Add 1 gram of fully-activated Florisil® to the column. Pre-wet the Florisil® with 1 mL of hexane and discard the eluant, if any. The wetted column is now ready for use.

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[10]There were marked differences in folpet elution characteristics between EPA's and Horizon's GPC columns; the superior resolution of EPA's column most likely relates to quality of the BioBeads S-X3 and improved column packing techniques.

[11]The EPA was able to validate this method at all fortification levels in avocados without using post-GPC clean-up techniques[4]. However, supplemental Florisil® and C-18RP clean-up techniques were required during HORIZON's independent laboratory validation (ILV) study with avocados. Use of the optional techniques is most likely matrix- and variety-within-matrix dependent; optional techniques are indicated if post-GPC sample chromatography is highly complex and/or analyte recoveries are grossly biased.

b. Dissolve the post-GPC dry residue from D.4.d in 5 mL of hexane. Transfer the entire sample to the top of the Florisil® column, taking care not to disturb the column surface. Gravity-percolate the sample into the column (ca. 2 drops/second), then wash the column sides with 6 mL of hexane. Percolate the rinse through the column. Discard all eluants to this point (Note 6, p. 24).

c. Elute folpet from the column with 5 mL of DCM containing 1% methanol (Note 7, p. 24) into a 25 mL evaporation flask.

d. Vacuum-evaporate (<35°C) the eluant to just-dryness. Dissolve the dry residue in a known volume of 2% DEGDEE in hexane (for LOQ residues, generally 6 mL). Dilute with the same solvent as necessary to maintain the analyte concentration within the standard curve range. Submit the sample for GC/ECD analysis as described in Step E.

If Florisil® column chromatography yields unacceptable sample clean-up, proceed to step D.6 below.

D.6 C-18RP Chromatography: Oily Crops (Optional)[11]

a. Beginning with the extract from D.5.c, vacuum-evaporate (<35°C) the eluant to just-dryness. Dissolve the residue in 2 mL of ACN (with sonication and swirling), then add 3 mL of water and mix (Note 8, p. 25).

b. Prepare a C-18RP SPE cartridge as follows: Place the cartridge onto a commercial manifold vacuum system. Wash the column with 2 x 5 mL of ACN, then 2 x 5 mL of water, by pulling a vacuum sufficient to elute the column at ca. 5 mL/minute. **Do not let the column go dry at any point in this procedure.** The column is now ready for use.

c. Percolate the entire sample from D.6.a through the column with vacuum (1 → 2 mL/minute). Wash the column with 15 mL of 40% ACN:water (v:v); discard all eluants to this point. **Do not let the column go dry at any point in this procedure.**

d. Elute folpet from the column with 15 mL of 60% ACN:water (v:v) into a 50 mL evaporation flask (Note 9, p. 25).

e. Vacuum-evaporate (<35°C) the eluant to dryness. Dissolve the dry residue in a known volume of 2% DEGDEE in hexane (for LOQ residues, generally 6 mL). Dilute with the same solvent as necessary to maintain the analyte concentration within the standard curve range. Submit the sample for GC/ECD analysis as described in Step E.

E. Gas Chromatographic Analysis

E.1 Equipment

A gas chromatograph equipped with an Electron Capture Detector is required. Split-splitless injection and mega-bore column capabilities are suggested.

GC Column: DB-1, 30 M length, 0.53 mm i.d., 1.5 μM film thickness, J & W Scientific, 91 Blue Ravine Road, Folsom, CA, Catalog #125-1032. Other columns may be substituted if they give satisfactory resolution between the folpet analyte and any interferences.

E.2 Suggested Operating Conditions: ILV[12]

Injector: 210°C, 2 mm dia. open glass insert.

Detector: 300°C, ECD.

Column:

Initial:	200°C, hold 10 min.
Ramp Rate 1:	40°C/min to 280°C, hold 3 min.

Carrier Gas: He, 9.5 mL/min at 200°C, head pressure = 10.0 psi. Constant flow off.

Septum Purge: He @ 3.6 mL/min.

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[12]Suggested in the ILV study[3].

Split Vent:	He @ 57 mL/min, on @ 0.75 min.
Detector make up:	N_2 @ 60 mL/min.
Injection:	1 µl, Split/Splitless

E.3 Suggested Operating Conditions: EPA[13]

Injector: 210°C

Detector: 300°C, ECD

Column:

Initial:	200°C, hold 10 min.
Ramp Rate 1:	40°C/min to 280°C, hold 30 min.

Carrier Gas:	He, 10.1 mL/min
Detector make up:	50 mL/min, 95% Argon:5% methane

F. Calibration Procedures

F.1 Preparation of Standard Solutions

a. Stock solutions of folpet are made in acetone and are stored in a freezer; stock concentrations are approximately 1.0 mg/mL. These solutions are stable for at least 3 months when stored in a freezer[3].

b. Dilutions of folpet stock solutions are made at appropriate concentrations for fortification standards (e.g., for a 25 gram sample and a 0.5 mL standard aliquot, 2.5, 50.0, and 500 µg/mL for 0.05, 1.0, and 10 ppm fortifications, respectively). These dilutions are made in EtOAc and stored in a freezer. These solutions are stable for at least 3 months when stored in a freezer[3].

c. Dilutions of folpet stock solutions are made at appropriate concentrations for GC/ECD assay standards (e.g., 0.0125 to 0.30 µg/mL). These dilutions are made in hexane containing 2%

[13]Suggested by EPA[4].

DEGDEE and are stored in a freezer. These solutions are stable for at least 3 months when stored in a freezer[3].

In some laboratories[3,4], folpet standards prepared in neat hexane gave unacceptable chromatography; standard curves exhibited poor linearity due to degradation of folpet into unknown components during chromatography. This degradation was characterized by time-dependent appearance of two, early-eluting, unknown degradation products plus concomitant loss of folpet signal. "Priming" the GC with UTC extracts exacerbated the problem. Techniques such as cool and hot on-column injection, temperature programming, and other column types were attempted with limited success. Use of the 2% DEGDEE in hexane solvent for GC standards and samples virtually eliminated these problems and improved the signal/noise ratio for folpet detection at all concentrations. The mechanism of action for this effect is not known.

F.2 Detector Calibration

The sensitivity of the ECD detector is monitored by injecting folpet standards before, between, and after the samples. The suggested mass range is 12.5 pg to 300 pg injected. Folpet must be detectable at the chosen minimum concentration. A standard of 12.5 pg folpet injected (1.0 µL of a 0.0125 µg/mL standard) and a final dilution volume of (a) 4 mL for a 25 gram non-oily crop sample, or (b) 6.0 mL for a 25 gram oily crop sample, results in calculated limits of quantification (LOQ) of 0.025 ppm and 0.0225 ppm folpet, respectively.

Under the conditions of the assay presented in Section E.2, folpet elutes from the GC column at ca. 9.2 minutes. The GC/ECD limit of detection for folpet (injected as a solution of pure standard) is 2 → 5 pg injected.

G. Methods of Calculation

G.1 Injection Sequence

Run sequences are started and ended with one or two standards; standard injections are made throughout the run, generally with no more than two sample

injections between each standard. A standard curve of folpet concentration (µg/mL) versus peak height or area is constructed using a method of curve generation appropriate for the GC/ECD instrumentation. The construction may be linear, quadratic or logarithmic.

G.2 Calculations

Calculate ppm values for folpet residues using the following equations:

For apples, cantaloupe, cranberries, cucumbers, grapes, lettuce, strawberries, and tomatoes:

$$ppm = \frac{(ug/mL\ Final\ Extract)\ X\ (mL\ Final\ Extract)}{Initial\ Extract\ Volume\ (mL)} \times \frac{Pre\text{-}Florisil\ Volume\ (mL)}{Aliquot\ Extract\ Volume\ (mL)} \times \frac{1}{Florisil\ Aliquot\ Volume\ (mL)} \times \frac{1}{25\ grams}$$

For onions:

$$ppm = \frac{(ug/mL\ Final\ Extract)\ X\ (mL\ Final\ Extract)}{Initial\ Extract\ Volume\ (mL)} \times \frac{Pre\text{-}Florisil\ Volume\ (mL)}{Aliquot\ Extract\ Volume\ (mL)} \times \frac{Post\text{-}Florisil\ Volume\ (mL)}{Florisil\ Aliquot\ Volume\ (mL)} \times \frac{1}{C\text{-}18RP\ Aliquot\ Volume\ (mL)} \times \frac{1}{25\ grams}$$

For avocados:

$$ppm = \frac{(ug/mL\ Final\ Extract)\ X\ (mL\ Final\ Extract)}{Initial\ Extract\ Volume\ (mL)} \times \frac{Pre\text{-}GPC\ Volume\ (mL)}{Aliquot\ Extract\ Volume\ (mL)} \times \frac{Pre\text{-}Florisil\ Volume\ (mL)}{GPC\ Aliquot\ Volume\ (mL)} \times \frac{Post\text{-}Florisil\ Volume\ (mL)}{Florisil\ Aliquot\ Volume\ (mL)} \times \frac{1}{C\text{-}18RP\ Aliquot\ Volume\ (mL)} \times \frac{1}{25\ grams}$$

The following example calculations use a log/log model for expressing the relationship between the concentration of folpet injected into the GC versus the ECD height response.

Example Calculation, Tomatoes: For a 1.0 ppm folpet fortification to tomatoes, the sample response is 78034 height units. The equation of the associated standard curve [log (height response)

versus log (µg folpet/mL)] is log Y = (0.92474)(log X) + 5.53436, where Y is the peak height and X is µg folpet/mL in the final extract. Solving for X with the 78034 unit response yields 0.2021 µg/mL folpet in the final extract. The final extract volume is 10 mL. The initial extract volume was 250 mL, from which a 50 mL aliquot volume was taken for further analysis. The sample volume before Florisil® chromatography was 5 mL; a 2 mL aliquot was cleaned-up with Florisil®. The original sample mass was 25.0 grams. Hence, ppm folpet in this tomato sample is (0.2021 ng/mL) x (10 mL) x (250 mL/50 mL) x (5 mL/2 mL) x (1/25 g) = 1.0107 ppm.

Example Calculation, Onions: For a 0.05 ppm folpet fortification to onions, the sample response is 8747 height units. The equation of the associated standard curve [log (height response) versus log (µg folpet/mL)] is log Y = (0.88604)(log X) + 5.44659. Solving for X with the 8747 unit response yields 0.0200 µg/mL folpet in the final extract. The final extract volume is 4 mL. The initial extract volume was 250 mL, from which a 50 mL aliquot volume was taken for further analysis. The sample volume before Florisil® chromatography was 5 mL; a 2 mL aliquot was cleaned-up with Florisil®. The entire 5 mL post-Florisil® sample was taken for further analysis; hence, the C-18RP aliquot volume was 5 mL. The original sample mass was 25.0 grams. Thus, ppm folpet in this onion sample is (0.0200 ng/mL) x (4 mL) x (250 mL/50 mL) x (5 mL/2 mL) x (5 mL/5 mL) x (1/25 g) = 0.0401 ppm.

Example Calculation, Avocados: For a 10.0 ppm folpet fortification to avocados, the sample response is 45981 height units. The equation of the associated standard curve [log (height response) versus log (µg folpet/ mL)] is log Y = (0.89298) (log X) + 5.44788. Solving for X with the 45981 unit response yielded 0.1320 µg/mL folpet in the final extract. The final extract volume is 200 mL. The initial extract volume was 250 mL, from which a 50 mL aliquot volume was taken for further analysis. The sample volume before GPC chromatography was 7.5 mL; the GPC aliquot volume was 5 mL. The entire post-GPC sample was cleaned-up on Florisil®; hence, the pre-Florisil® extract volume divided by the Florisil® aliquot volume was 1. The entire post-Florisil® sample was taken through C-18RP chromatography, hence, the post-Florisil® volume divided by the C-18RP aliquot volume was 1. The

original sample mass was 25.0 grams. Thus, ppm folpet in this avocado sample is (0.1320 ng/mL) x (200 mL) x (250 mL/50 mL) x (7.5 mL/5 mL) x (1) x (1) x (1/25 g) = 7.920 ppm.

Recovery of folpet from fortified samples is calculated according to the following formula:

$$\% \ Recovery = \frac{(ppm\ found) - (ppm\ control)}{ppm\ fortified} \times 100$$

For example, recovery of folpet from the avocado sample described above is [(7.920 ppm - 0.013 ppm[14])/10 ppm] x 100 = 79.1%

H. Interferences

H.1 Sample Matrices

Chromatographic interferences are generally inconsequential in extracts from apple, cantaloupe, cucumber, grape, strawberry, and tomato samples. Extracts from cranberry and lettuce samples contain several small chromatographic peaks, but none near the retention time of folpet. In the ILV study[3], avocado and onion extracts exhibited highly complex chromatography which compromised the analysis unless supplemental clean-up steps were employed (i.e., Florisil® and/or C-18RP column chromatography). However, these additional clean-up steps were not required for avocado analysis by the EPA[4]. For all matrices, apparent residues in control matrices have been generally non-detectable and, when present, have always been less than the 0.05 ppm LOQ.

Nonetheless, analysts using these methods should carefully examine each sample/standard chromatogram and judge the suitability of the sample clean-up techniques which were employed.

Despite the clean-up procedures employed in these methods, extensive sample injections do cause some undesirable chromatographic effects, most notably

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[14]There were two controls analyzed parallel with this sample. Folpet measured in one control was 0.026 ppm; in the other control, folpet was non-detectable. Hence, the average control residue was 0.013 ppm.

reduced instrument sensitivity towards folpet and loss of resolution from interferences. This problem is corrected by cleaning the injector insert and cutting 10 → 20 cm from the proximal end of the column.

H.2 Other Pesticides

A specificity study has been conducted for this method[1].

H.3 Solvents and Reagents

The solvents and reagents specified in this procedure do not present any interferences at the stated LOQ.

H.4 Glassware

No interferences are detected from the labware at the stated LOQ. Glassware is pre-rinsed with acetone, then dried prior to use. Glass vessels are recommended for all steps without substitutions with plastic.

III. METHODS VALIDATION

The enforcement methodology described in this document incorporates elements from four sources[1,2,3,4]. The original "non-oily" crop method[1] was tested against a variety of crops fortified at multiple levels with folpet. The original "oily" crop method[2] evaluated the analysis of avocados at multiple fortification levels. Both methods were independently validated (with modifications) in a third study[3], then validated at the EPA[4]. A summary of all results is presented in Table 1.

Briefly, the mean recovery of folpet measured in a variety of fortified crop sources was 95% (N = 164 samples). Fortification levels ranged from 0.050 ppm to 50.3 ppm, depending upon the matrix. Considering all laboratories, the standard deviation for folpet recovery ranged from 2.8% (apples) to 19% (melons) of the mean. Hence, the methodology shows excellent ruggedness and is reasonably independent of matrix and fortification level.

The time required for analysis of 8 samples by one chemist ranges from 12.5 to 17.5 hours (over 2 days), depending upon the matrix and laboratory[1,2,3,4].

IV. NOTES

Note 1: Multiple aliquots of untreated control matrix for quality control purposes may be placed in plastic weigh boats and held on wet ice pending fortification and extraction. However, unused portions of homogenized field samples have no further analytical value and should be discarded as waste. The instability of folpet in frozen and thawed macerated crops is well documented[15] and is attributed to enzymatic activity endogenous to the sample.

Note 2: Addition of phosphoric acid is a critical step in this procedure since folpet is unstable at an alkaline pH.

Note 3: Other receiving vessels may be used. Graduated cylinders with standard-taper openings attached to vacuum take-offs are recommended, as these containers facilitate volume measurements and permit mixing of the resulting extract.

Note 4: Phase separation is rapid and without emulsions.

Note 5: The pre-wash with EtOAc removes potential interferences from the sodium sulfate. Use the highest available grade of anhydrous sodium sulfate, preferably one that has been ignited by the manufacturer at temperatures greater than 600°C.

Note 6: The pre-wash with hexane elutes several matrix-related chromatographic interferences from the sample.

Note 7: Fractionation parameters for each batch of Florisil® must be independently evaluated. Prepare a Florisil® column as noted in C.4.a. Also prepare a 0.40 µg/mL folpet solution in hexane. Pipet 2 mL of this solution onto the column and process the sample as noted in steps C.4.b through C.4.d (Note: retain all washes in C.4.b separately to this point) with a 4 mL final sample volume. Inject the final sample into the GC/ECD (see Section E) against a 0.20 µg/mL folpet solution prepared in 1% DEGDEE:hexane (see Section F.1). At 100% recovery, the GC/ECD detector responses for the two injections should be equivalent.

If the recovery is biased low, vacuum-evaporate the wash from C.4.b, dissolve the residue in 2 mL of 1% DEGDEE: hexane, then inject into the GC/ECD. Similarly, elute the column with additional 1% methanol in DCM, vacuum-evaporate to just-dryness, dissolve the residue, then

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[15]Personal communication by the author with the study sponsor.

inject into the GC/ECD. Adjust the column elution parameters accordingly to collect the folpet residue quantitatively.

Commercial Florisil® columns may also be used. The original Analyst Ltd. method[1] recommends the use of 1 gram columns prepared by Varian (Part #1211-3049, solid-phase Bond-Elute cartridges). As with the Florisil® columns prepared in the laboratory, each lot of commercial columns must be independently evaluated as described above.

Note 8: ACN is added to dissolve the residue, then water is added to yield a 40% ACN:water solution. Poorly-soluble matrix interferences inhibit quantitative dissolution of folpet if the 40% ACN:water is added as a complete solution.

Note 9: Fractionation parameters for each lot of commercial C-18RP columns must be independently evaluated. Prepare a C-18RP column as noted in C.5.b. Also prepare a 0.40 µg/mL folpet solution in 40% ACN:water. Pipet 5 mL of this solution onto the column and process the sample as noted in steps C.5.c through C.5.e (Note: retain all washes in C.5.c separately to this point) with a 10 mL final sample volume. Inject the final sample into the GC/ECD (see Section E) against a 0.20 µg/mL folpet solution prepared in 1% DEGDEE:hexane (see Section F.1). At 100% recovery, the GC/ECD detector responses for the two injections should be equivalent.

If the recovery is biased low, vacuum-evaporate the wash from C.5.c, dissolve the residue in 2 mL of 1% DEGDEE: hexane, then inject into the GC/ECD. Similarly, elute the column with additional 60% ACN:water, vacuum-evaporate to just-dryness, dissolve the residue, then inject into the GC/ECD. Adjust the column elution parameters accordingly to collect the folpet residue quantitatively.

Note 10: The sodium sulfate aids dissolution of the residue by scouring the sides of the evaporation flask and suspending slowly-soluble materials.

Note 11: The GPC column is prepared by swelling ca. 40 grams of BioRad BioBeads S-X3 (200-400 mesh) overnight in DCM:acetone (3:7, v:v). The column is packed and purged at 5 mL/minute with the same solvent until air bubbles are absent. The column is compressed by adjusting the column end-fittings, and all sample loops are purged with eluting solvent to eliminate air from the system (**Note: Disconnect the GPC column from the instrument before purging air from the sample loops**).

Each GPC column must be calibrated prior to use. Prepare 7.5 mL of a ^{14}C-folpet solution (ca. 1 µg/mL, containing at least 0.25 µCi of ^{14}C) and inject this solution into the GPC (5 mL sample loop). Elute the column with the DCM:acetone (3:7, v:v) solvent and collect 10 mL fractions. Assay each fraction for ^{14}C content by conventional scintillation analysis to define the elution profile for folpet. This calibration may also be conducted with non-radioactive folpet, except that each 10 mL fraction must be vacuum-evaporated to just-dryness, dissolved in 2% DEGDEE in hexane (e.g., 2 mL, see Section F.1), then assayed by GC/ECD (see Section E).

V. TABLES

Table 1. Recovery of Folpet Residues from Various Crop Matrices.

MATRIX	FORTIFICATION LEVEL (PPM)	NUMBER OF SAMPLES	SOURCE	MEAN RECOVERY (%)	STANDARD DEVIATION (%)
Apples	0.050-2.50	9	(a)	99	2.8
	0.050-10.0	6	(c)	104	3.1
Avocados	0.050-10.0	6	(b)	101	9.9
	0.050-10.0	6	(c)	76	8.8
	0.052-25.2	6	(d)	94	9.4
Cranberries	0.050-10.0	6	(c)	103	13
Grapes	0.050-5.00	8	(a)	94	8.9
	0.050-10.0	6	(c)	106	4.5
Lettuce	0.050-50.0	12	(a)	93	7.2
	0.050-10.0	6	(c)	92	4.8
	0.052-50.3	6	(d)	87	8.3
Melons Cantaloupe	0.050-2.50	9	(a)	95	18
	0.050-10.0	6	(c)	103	3.8
Onions	0.050-10.0	6	(c)	87	13
	0.052-15.1	6	(d)	82	8.8
Squash Cucumbers	0.050-2.50	9	(a)	89	15
	0.050-10.0	6	(c)	95	2.8
Potatoes	0.050-2.50	9	(a)	92	13
Strawberries	0.050-2.50	9	(a)	95	12
	0.050-10.0	6	(c)	106	13
Tomatoes	0.050-2.50	9	(a)	100	16
	0.050-10.0	6	(c)	101	7.0
	0.052-26.2	6	(d)	106	5.2
		N = 164		Mean = 95	

(a) "A Method for the Determination of Folpan and Phthalimide Residues in Non-Oily Crops", by H. M. Schlesinger, Makhteshim Chemical Works Ltd., Beersheva, Israel, March 4, 1992.

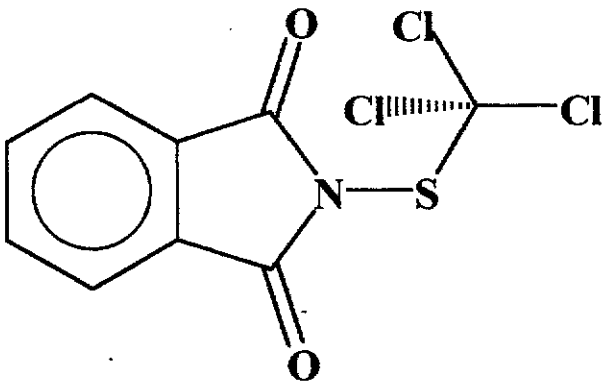
(b) "A Method for the Determination of Folpet Residues in Avocados and Other Oily Crops", by L. T. Nishioka, et. al., PTRL West, Inc., 4123-B Lakeside Drive, Richmond, CA, March 5, 1996.

(c) "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., 1610 Business Loop 70 West, Columbia, MO, June 4, 1996.

(d) "Validation of Folpet in: Avocados, Tomatoes, Lettuce, and Onions", a letter from A. J. Krynitsky (Chemist, EPA) to E. Zager, (Acting Chief, Chemistry Branch I/Tolerance Support, Health Effects Division, EPA), September 30, 1996.

VI. FIGURES

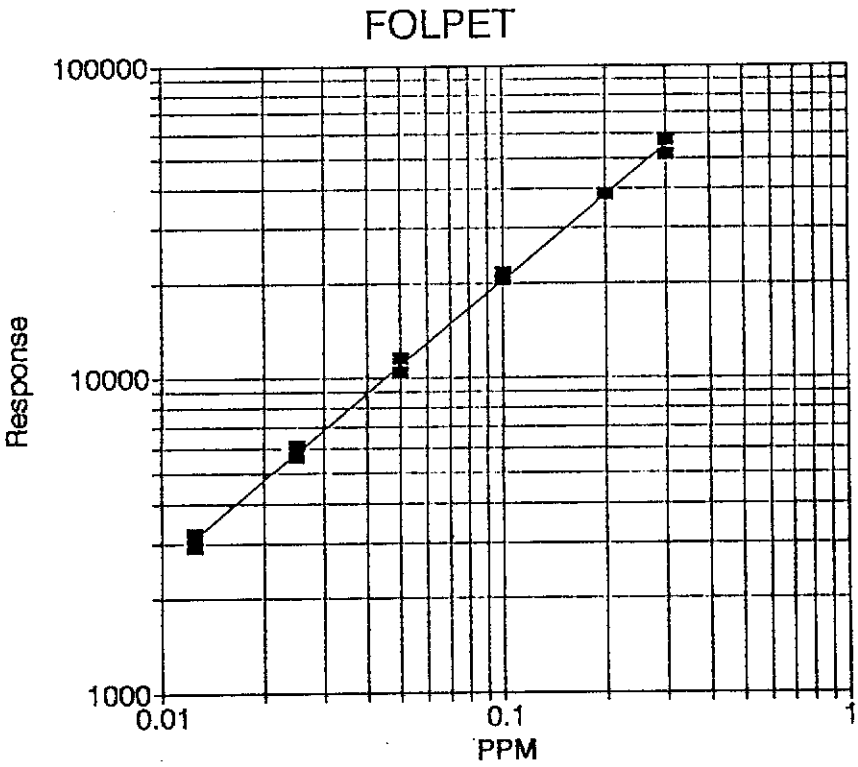
Figure 1. Chemical Structure of Folpet.



FOLPET N-[(trichloromethyl)thio] phthalimide

Figure 2. Example Chromatography

Example Standard Curve



Folpet Standard Curve \013196.wq1
For Lettuce, Trial 2

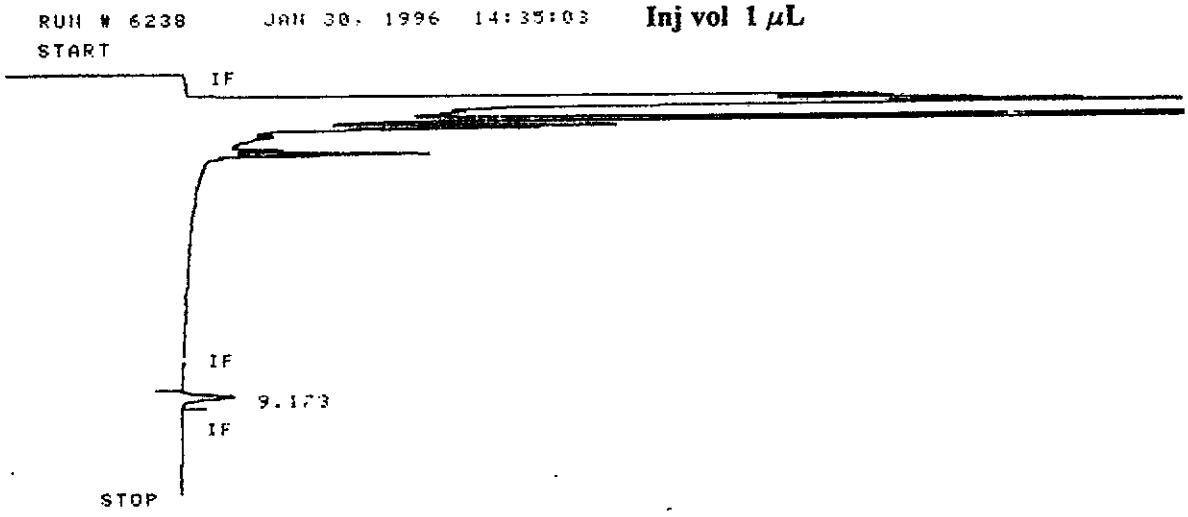
log(response) = 0.90575(log ppm[1]) + 5.21527

r = 0.9990

[1]ppm = µg/mL.

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #2 - Lettuce
0.0125 ppm Std GC012596.7 (Folpet)
Inj vol 1 μL



RUN# 6238 JAN 30, 1996 14:35:03

SAMPLE# 21

METHOD NAME: M*FOLPET2.MET

IDENTIFIER : GC#529
ESTD-AREA

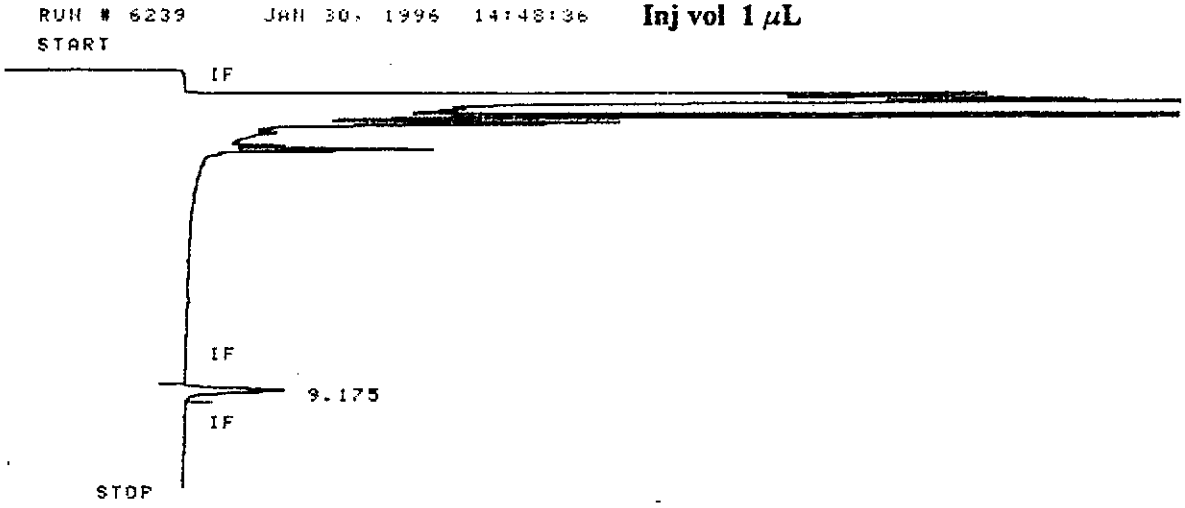
RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
9.173	PB	30610	.159	3204	1	.000	FOLPET

TOTAL AREA= 30610
MUL FACTOR=1.0000E+00

BEST AVAILABLE COPY

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #2 - Lettuce
0.025 ppm Std GC012596.6 (Folpet)
Inj vol 1 μL



RUN# 6239 JAN 30, 1996 14:48:36

SAMPLE# 22

METHOD NAME: M*FOLPET2.MET

IDENTIFIER : GC#529
ESTD-AREA

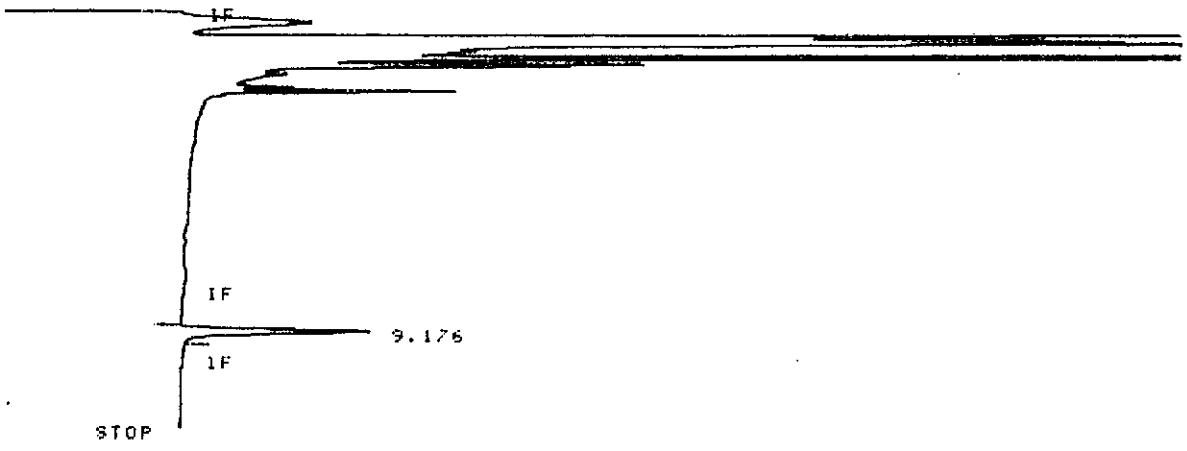
RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
9.175	PB	59954	.164	6085	1	.000	FOLPET

TOTAL AREA= 59954
MUL FACTOR=1.0000E+00

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #2 - Lettuce
0.05 ppm Std GC012596.5 (Folpet)
Inj vol 1 μL

RUN # 6241 JAN 30, 1996 15:15:39
START



RUN# 6241 JAN 30, 1996 15:15:39

SAMPLE# 24
METHOD NAME: M*FOLPET2.MET

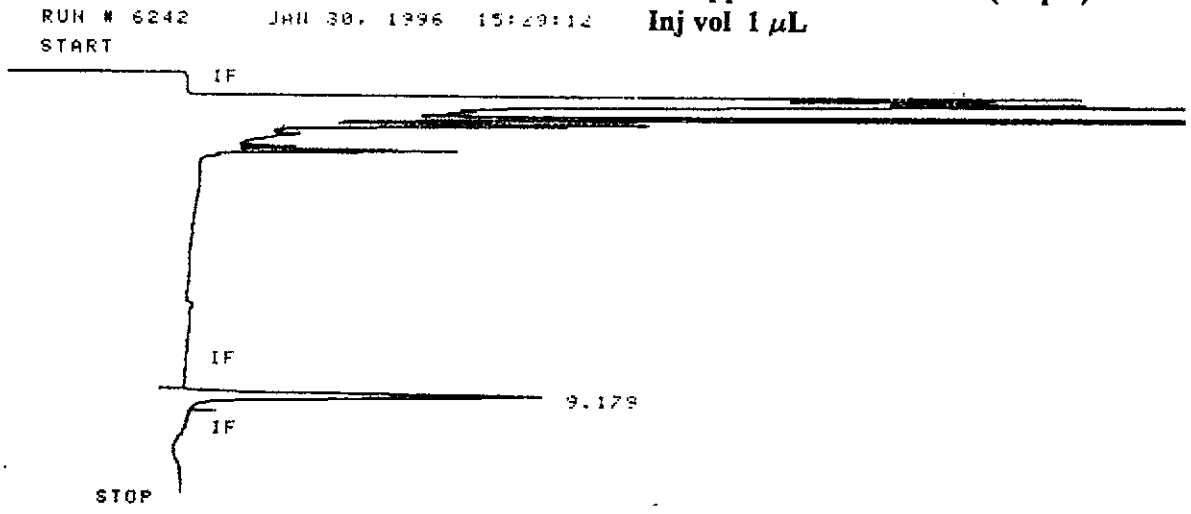
IDENTIFIER : GC#529
ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CHL#	AMOUNT	NAME
9.176	PB	113907	.166	11449	1	.000	FOLPET

TOTAL AREA# 113907
MUL FACTOR=1.0000E+00

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #2 - Lettuce
0.10 ppm Std GC012596.4 (Folpet)
Inj vol 1 μL



RUN# 6242 JAN 30, 1996 15:29:12

SAMPLE# 25

METHOD NAME: M*FOLPET2.MET

IDENTIFIER : GC#529

ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
9.179	PB	216175	.167	21625	1	.000	FOLPET

TOTAL AREA= 216175
MUL FACTOR=1.0000E+00

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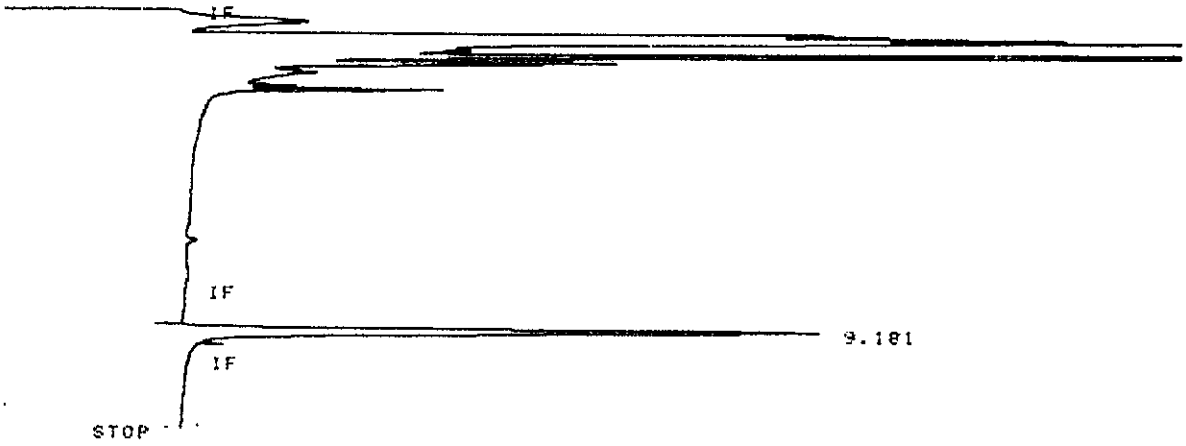
Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #2 - Lettuce
0.20 ppm Std GC012596.3 (Folpet)
Inj vol 1 μL



RUN# 6244 JAN 30, 1996 15:56:18

SAMPLE# 27

METHOD NAME: M*FOLPET2.MET

IDENTIFIER : GC#529
ESTD-AREA

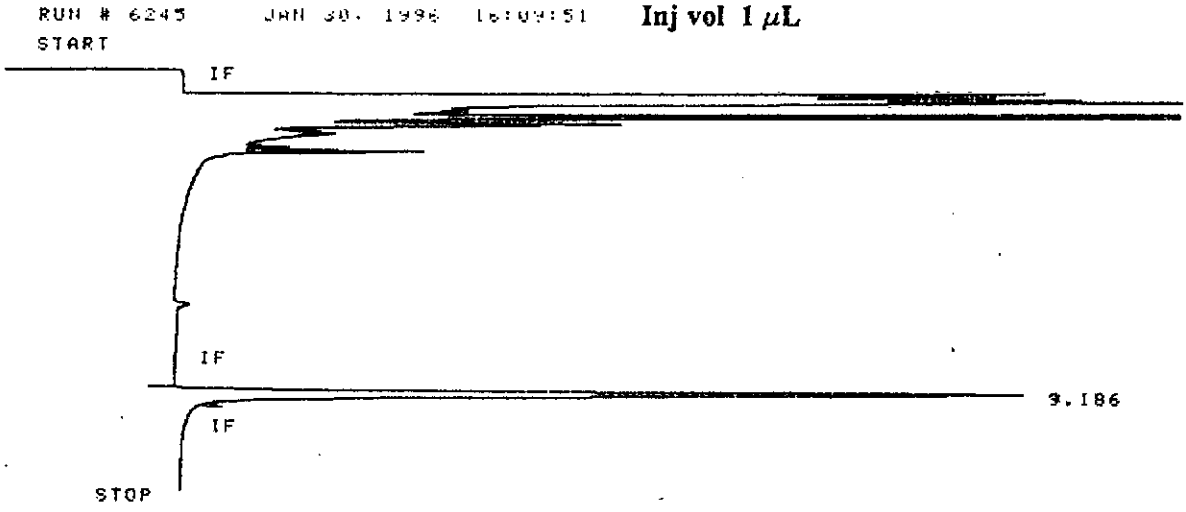
RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
9.181	PB	388362	.169	38413	1	.000	FOLPET

TOTAL AREA= 388362
MUL FACTOR=1.0000E+00

BEST AVAILABLE COPY

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #2 - Lettuce
0.30 ppm Std GC012596.2 (Folpet)
Inj vol 1 μL



RUN# 6245 JAN 30, 1996 16:09:51

SAMPLE# 28

METHOD NAME: M*FOLPET2.MET

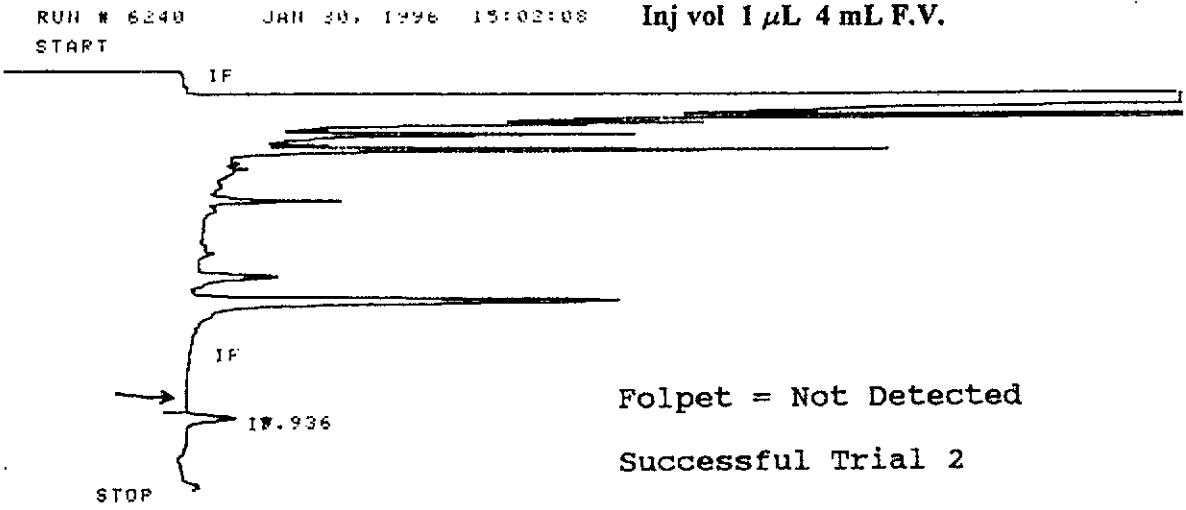
IDENTIFIER : GC#529
ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
9.186	PB	519114	.169	51092	1	.000	FOLPET

TOTAL AREA= 519114
MUL FACTOR=1.0000E+00

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #2 - Lettuce
10146-009 Control Lettuce
Inj vol 1 μL 4 mL F.V.



Folpet = Not Detected

Successful Trial 2

RUN# 6240 JAN 30, 1996 15:02:08

SAMPLE# 23

METHOD NAME: M*FOLPET2.MET

IDENTIFIER : GC#529
NO CALIB PEAKS FOUND
AREA%

RT	AREA	TYPE	WIDTH	AREA%
9.936	27069	I BH	.148	100.00000

TOTAL AREA= 27069
MUL FACTOR=1.0000E+00

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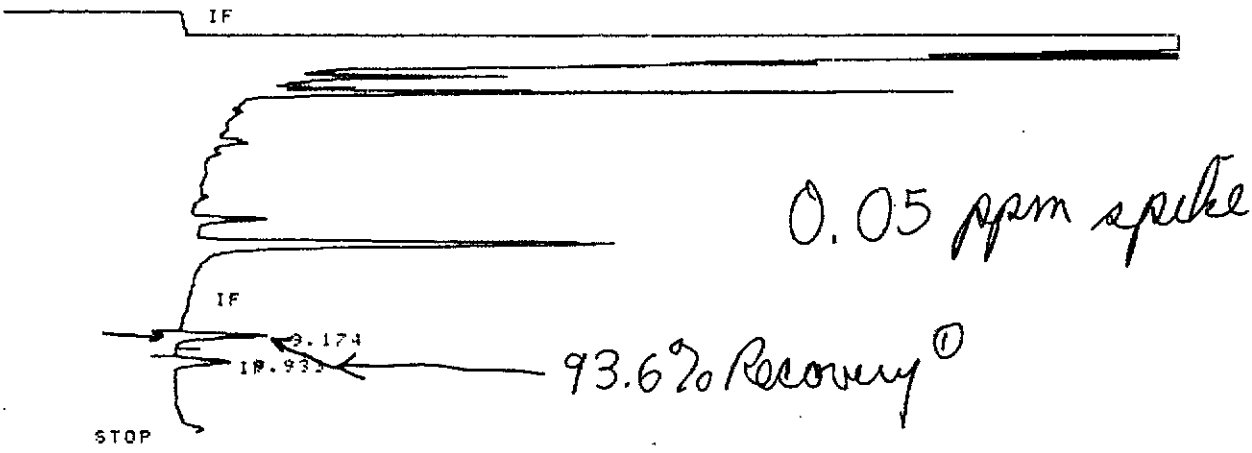
Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #2 - Lettuce
10146-011 Control Lettuce + 0.05 ppm
Inj vol 1 μL 4 mL F.V.

RUN # 6246 JAN 30, 1996 16:23:21
START



Folpet = 93.6% Recovery

Successful Trial 2

RUN# 6246 JAN 30, 1996 16:23:21

SAMPLE# 29

METHOD NAME: M*FOLPET2.MET

IDENTIFIER : GC#529
ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
9.174	BB	53258	.162	5472	1	.000	FOLPET
9.933	I BH	29603	.147	3350		.000	

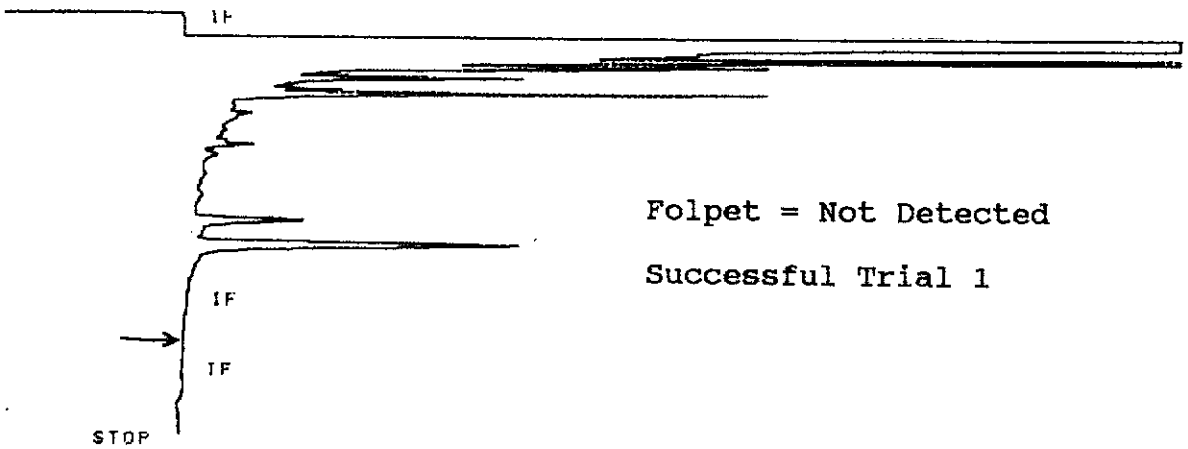
TOTAL AREA= 82861
MUL FACTOR=1.0000E+00

① I meant to write this on a copy, but inadvertently wrote on the original, MW, 1/30/96

BEST AVAILABLE COPY

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #1 - Grapes
10146-017 Control Grapes
Inj vol 1 μL 4 mL F.V.



Folpet = Not Detected

Successful Trial 1

RUN# 6220 JAN 30, 1996 10:30:32

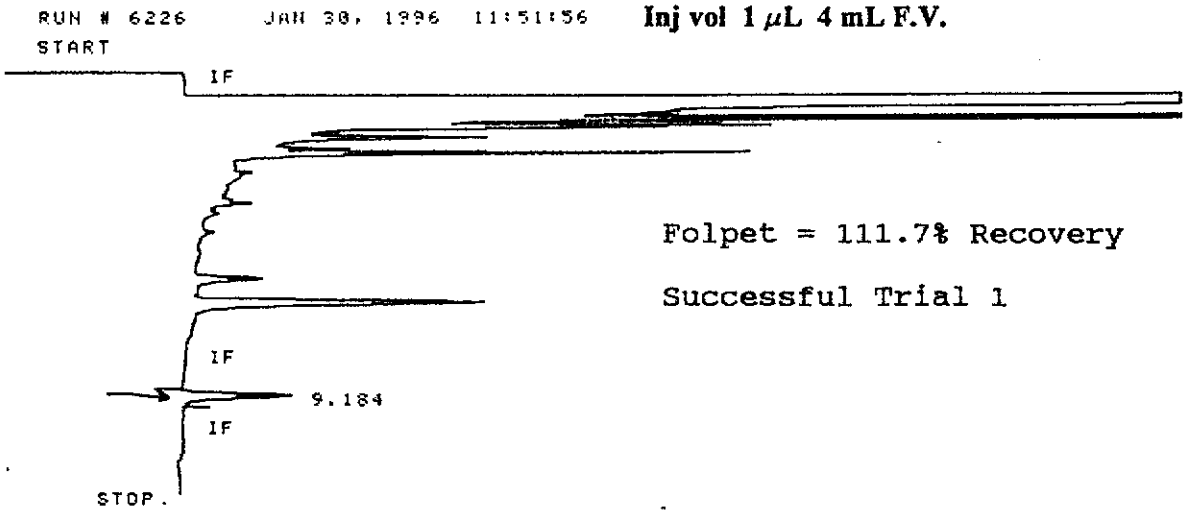
SAMPLE# 3

METHOD NAME: M*FOLPET2.MET

IDENTIFIER : GC#529
NO RUN PEAKS STORED

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #1 - Grapes
10146-019 Control Grapes + 0.05 ppm
Inj vol 1 μL 4 mL F.V.



RUN# 6226 JAN 30, 1996 11:51:56

SAMPLE# 9

METHOD NAME: M*FOLPET2.MET

IDENTIFIER : GC#529
ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
9.184	PB	65876	.165	6637	1	.000	FOLPET

TOTAL AREA= 65876
MUL FACTOR=1.0000E+00

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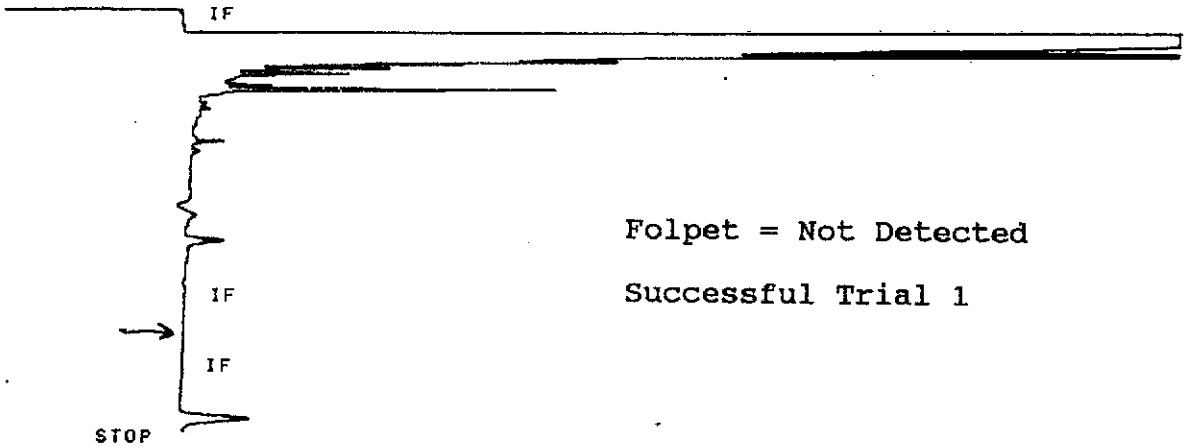
Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #1 - Cucumbers
10146-025 Control Cucumbers
Inj vol 1 μL 4 mL F.V.

RUN # 6340 FEB 13, 1996 14:59:17
START



Folpet = Not Detected

Successful Trial 1

RUN# 6340 FEB 13, 1996 14:59:17

SAMPLE# 3

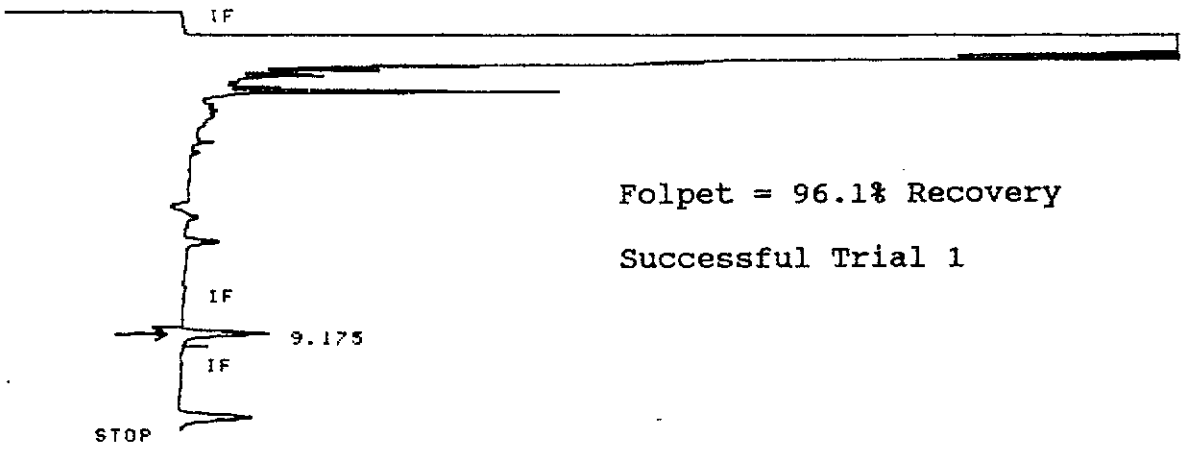
METHOD NAME: M*FOLPET2.MET

IDENTIFIER : GC#529
NO RUN PEAKS STORED

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #1 - Cucumbers
10146-027 Control Cucumbers + 0.05 ppm
Inj vol 1 μL 4 mL F.V.

RUN # 6346 FEB 13, 1996 16:20:42
START



Folpet = 96.1% Recovery

Successful Trial 1

RUN# 6346 FEB 13, 1996 16:20:42

SAMPLE# 9

METHOD NAME: M*FOLPET2.MET

IDENTIFIER : GC#529
ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
9.175	P8	101024	.159	10587	1	.000	FOLPET

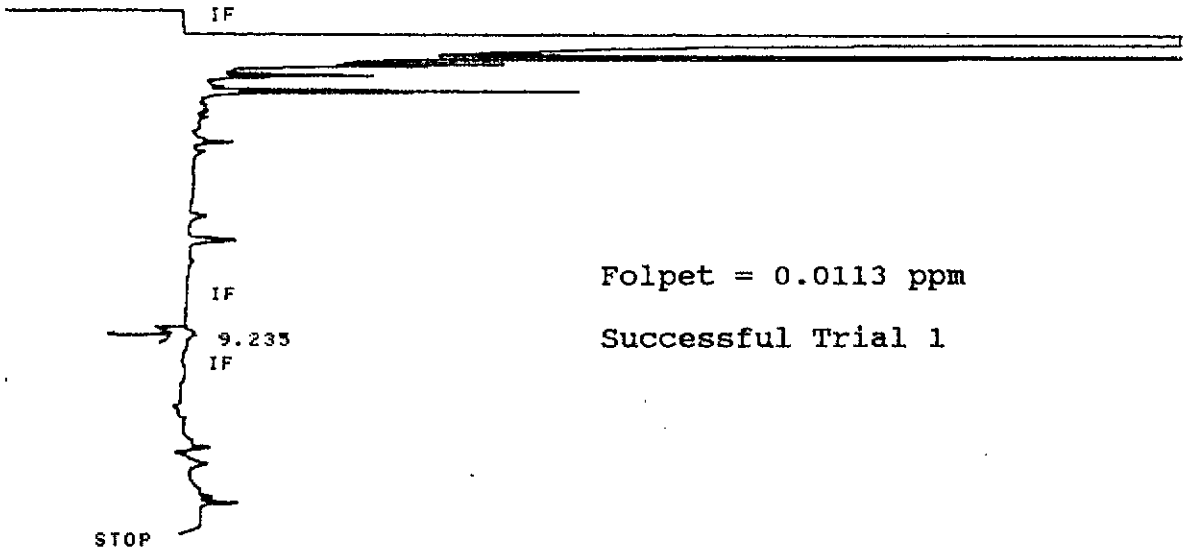
TOTAL AREA= 101024
MUL FACTOR=1.0000E+00

BEST AVAILABLE COPY

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #1 - Apples
10146-041 Control Apples
Inj vol 1 μL 4 mL F.V.

RUN # 6495 FEB 19, 1996 15:45:29
START



Folpet = 0.0113 ppm

Successful Trial 1

RUN# 6495 FEB 19, 1996 15:45:29

SAMPLE# 3

METHOD NAME: M*FOLPET2A.MET

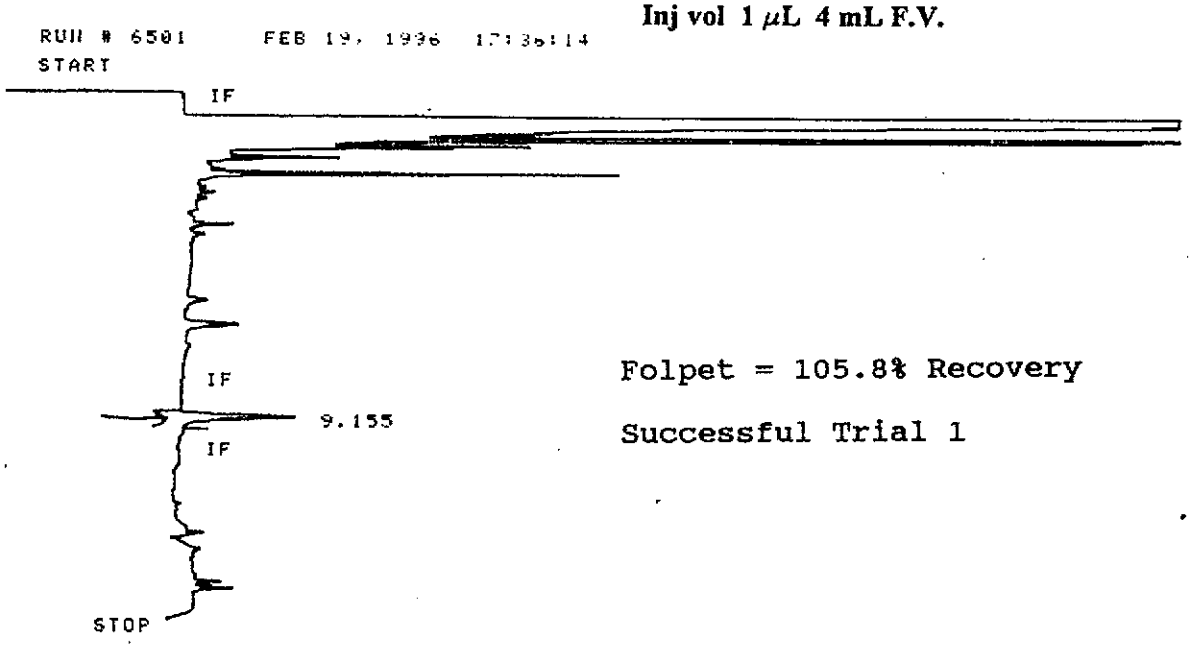
IDENTIFIER : GC#529
NO CALIB PEAKS FOUND
AREA%

RT	AREA	TYPE	WIDTH	AREA%	
9.235	26313	I BP	.299	100.00000	Folpet

TOTAL AREA= 26313
MUL FACTOR=1.0000E+00

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #1 - Apples
10146-043 Control Apples + 0.05 ppm
Inj vol 1 μL 4 mL F.V.



Folpet = 105.8% Recovery

Successful Trial 1

RUN# 6501 FEB 19, 1996 17:36:14

SAMPLE# 9

METHOD NAME: M*FOLPET2A.MET

IDENTIFIER : GC#529
ESTD-AREA

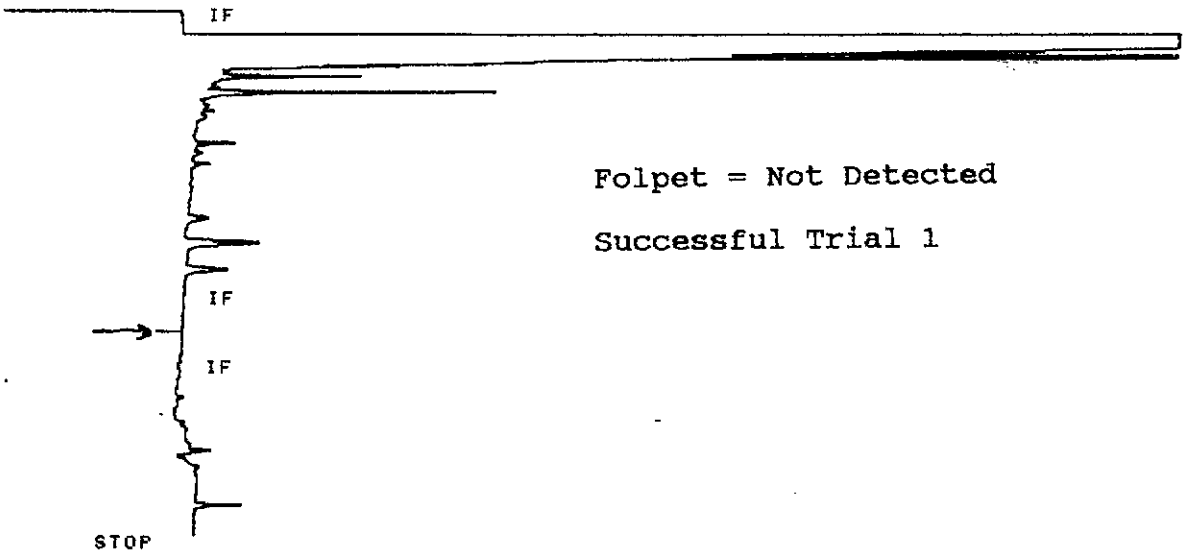
RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
9.155	PB	135169	.161	13956	1	.000	FOLPET

TOTAL AREA= 135169
MUL FACTOR=1.0000E+00

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #1 - Tomato
10146-049 Control Tomato
Inj vol 1 μL 4 mL F.V.

RUN # 6520 FEB 20, 1996 15:52:04
START



Folpet = Not Detected

Successful Trial 1

RUN# 6520 FEB 20, 1996 15:52:04

SAMPLE# 3

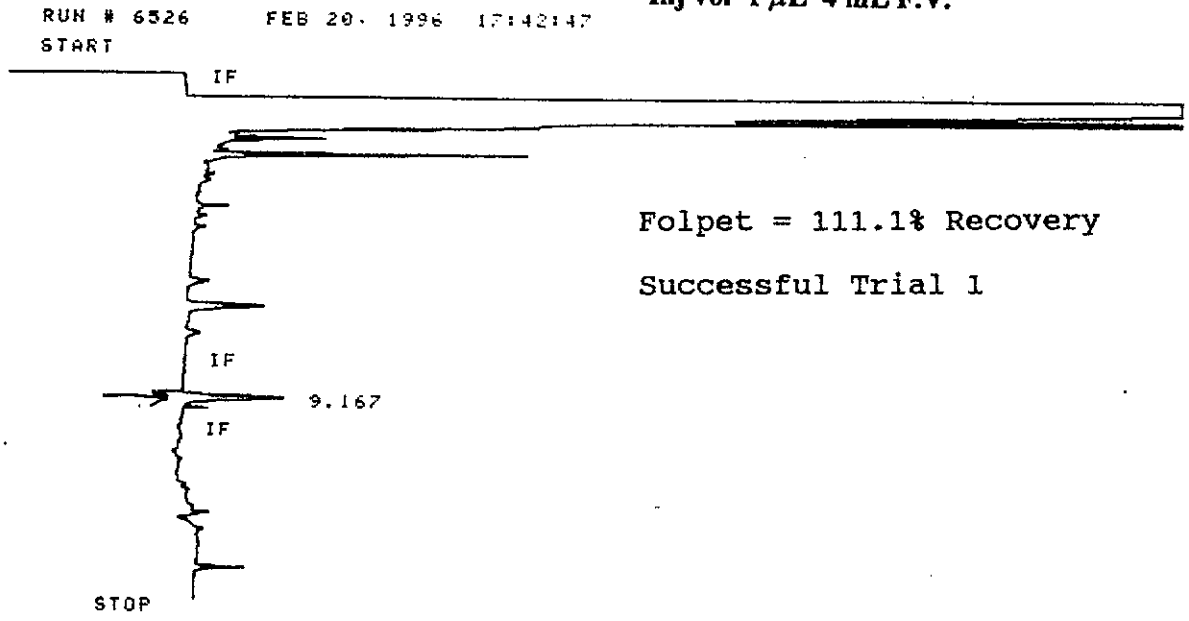
METHOD NAME: M*FOLPET2A.MET

IDENTIFIER : GC#529
NO RUN PEAKS STORED

BEST AVAILABLE COPY

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #1 - Tomato
10146-051 Control Tomato + 0.05 ppm
Inj vol 1 μL 4 mL F.V.



Folpet = 111.1% Recovery

Successful Trial 1

RUN# 6526 FEB 20, 1996 17:42:47

SAMPLE# 9

METHOD NAME: M*FOLPET2A.MET

IDENTIFIER : GC#529
ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
9.167	PB	115379	.154	12452	1	.000	FOLPET

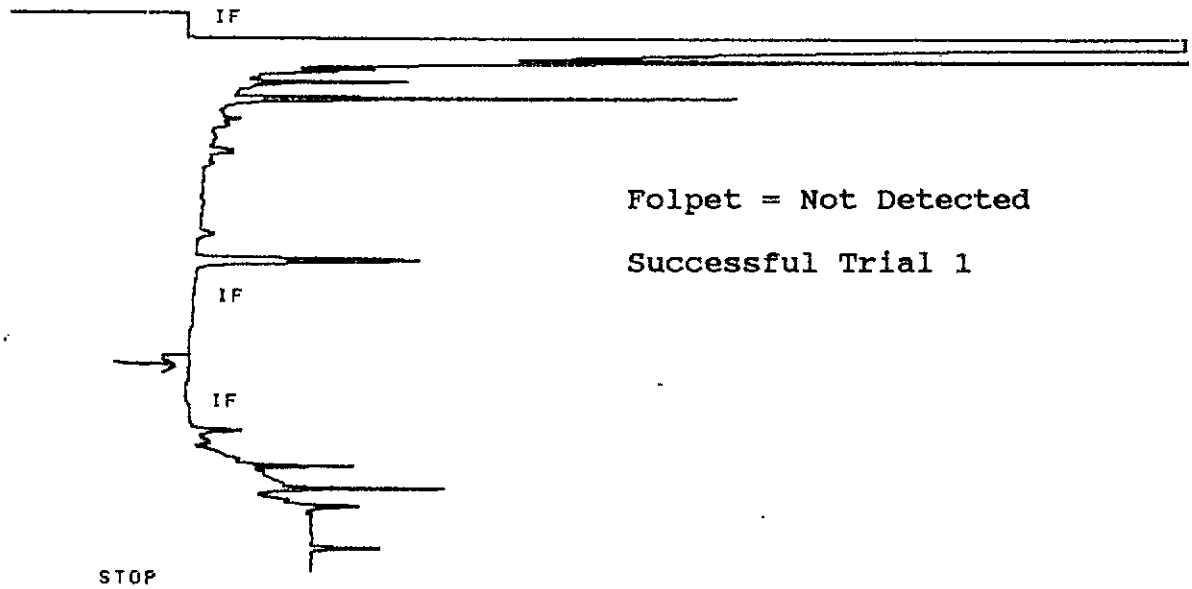
TOTAL AREA= 115379
MUL FACTOR=1.0000E+00

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #1 - Cantaloupe
10146-057 Control Cantaloupe
Inj vol 1 μL 4 mL F.V.

RUN # 1803-002

RUN # 1804 FEB 27, 1996 23:24:28
START



Folpet = Not Detected

Successful Trial 1

RUN# 1804 FEB 27, 1996 23:24:28

SAMPLE# 3

METHOD NAME: M*FOLPET2A.MET

IDENTIFIER : GC HL 526

~~BANANAS~~ (1)

NO RUN PEAKS STORED

(1)(E) REH 2-28-96

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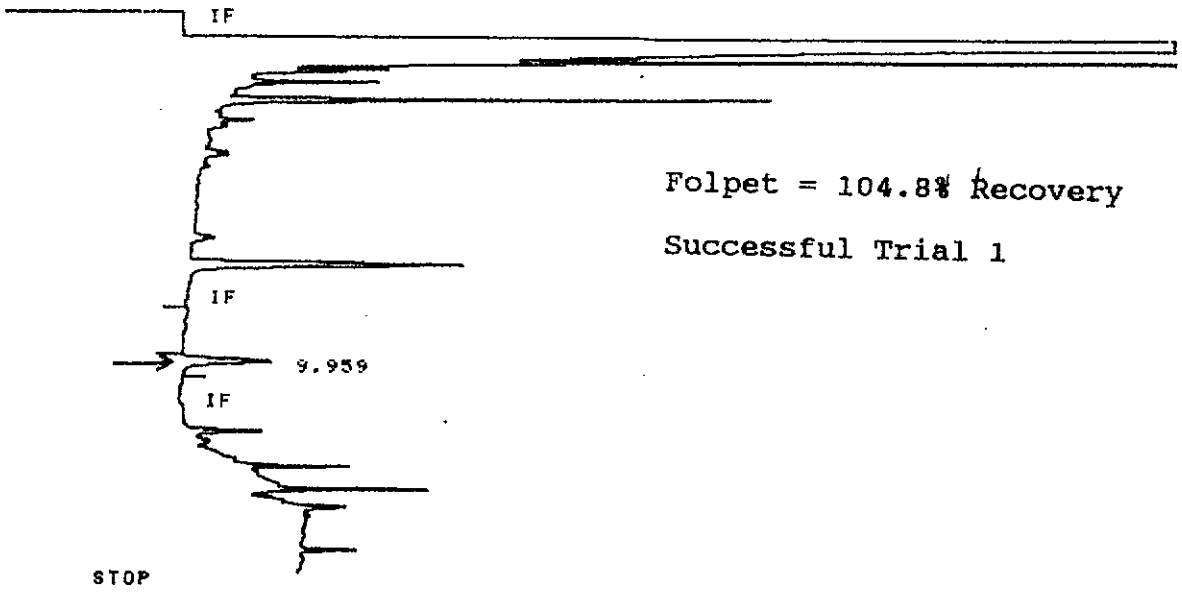
Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #1 - Cantaloupe
10146-059 Control Cantaloupe + 0.05 ppm
Inj vol 1 μL 4 mL F.V.

RUN # 1809-002

RUN # 1810 FEB 28, 1996 01:20:22
START



Folpet = 104.8% Recovery

Successful Trial 1

RUN# 1810 FEB 28, 1996 01:20:22

SAMPLE# 9

METHOD NAME: M*FOLPET2A.MET

IDENTIFIER : GC HL 526

~~BANANAS~~ ①

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ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CHL#	AMOUNT	NAME
9.959	PB	57277	.172	5536	1	.000	FOLPET

TOTAL HEIGHT= 5536
MUL FACTOR=1.0000E+00

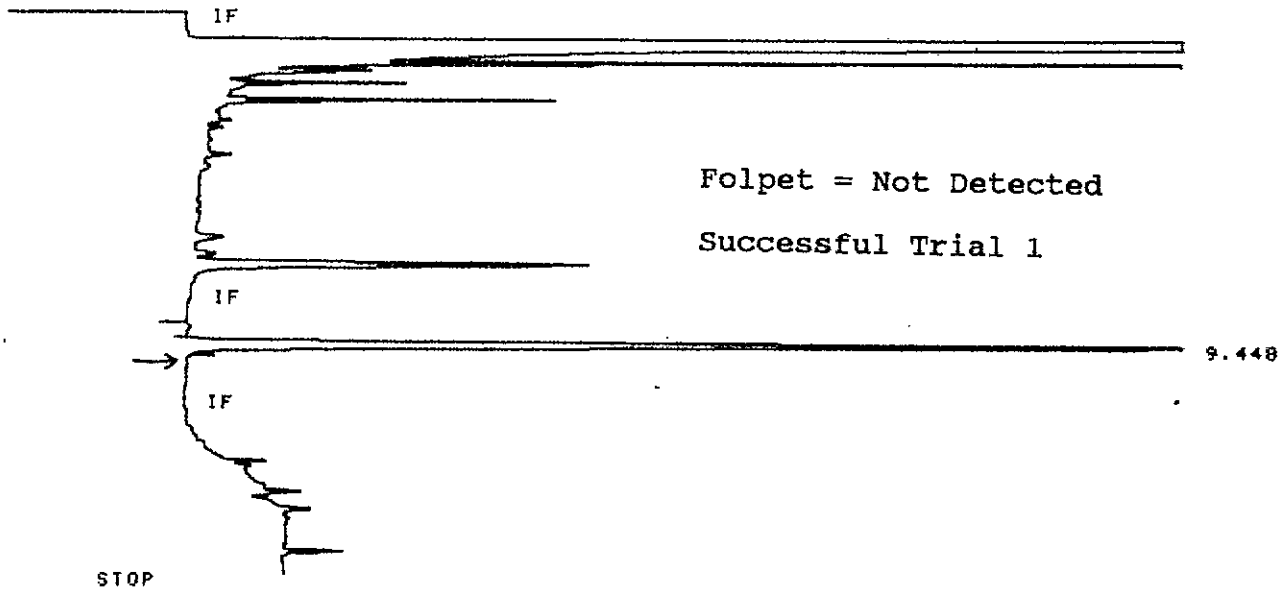
①② REH 2-28-96

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #1 - Strawberries
10146-065 Control Strawberries
Inj vol 1 μL 4 mL F.V.

RUN # 1823-002

RUN # 1824 FEB 28, 1996 05:50:26
START



RUN# 1824 FEB 28, 1996 05:50:26

SAMPLE# 23
METHOD NAME: M*FOLPET2A.MET

IDENTIFIER : GC HL 526

~~BANANAS~~ ①

NO CALIB PEAKS FOUND
HEIGHT%

RT	HEIGHT	TYPE	WIDTH	HEIGHT%
9.448	71237	PB	.152	100.00000

TOTAL HEIGHT= 71237
MUL FACTOR=1.0000E+00

①ⓔ MEH 2-28-96

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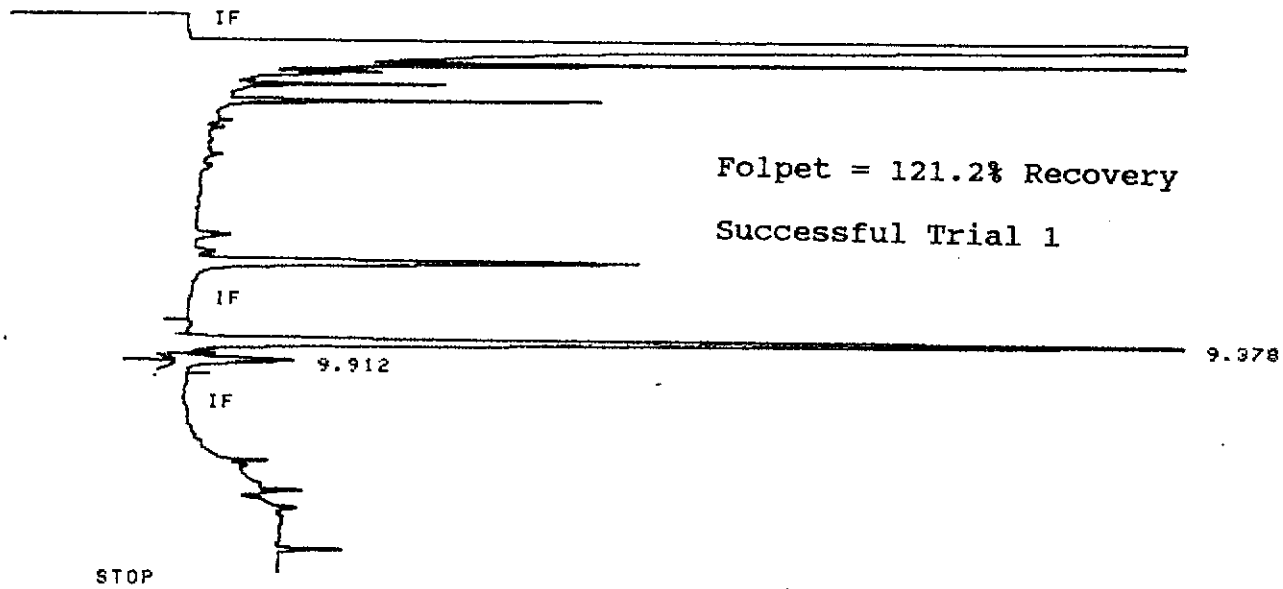
Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #1 - Strawberries
10146-067 Control Strawberries + 0.05 ppm
Inj vol 1 μL 4 mL F.V.

RUN # 1829-002

RUN # 1830 FEB 28, 1996 07:46:04
START



RUN# 1830 FEB 28, 1996 07:46:04

SAMPLE# 29

METHOD NAME: M*FOLPET2A.MET

IDENTIFIER : GC HL 526

~~BAHAMAS~~ ⓪

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ESTD-HEIGHT

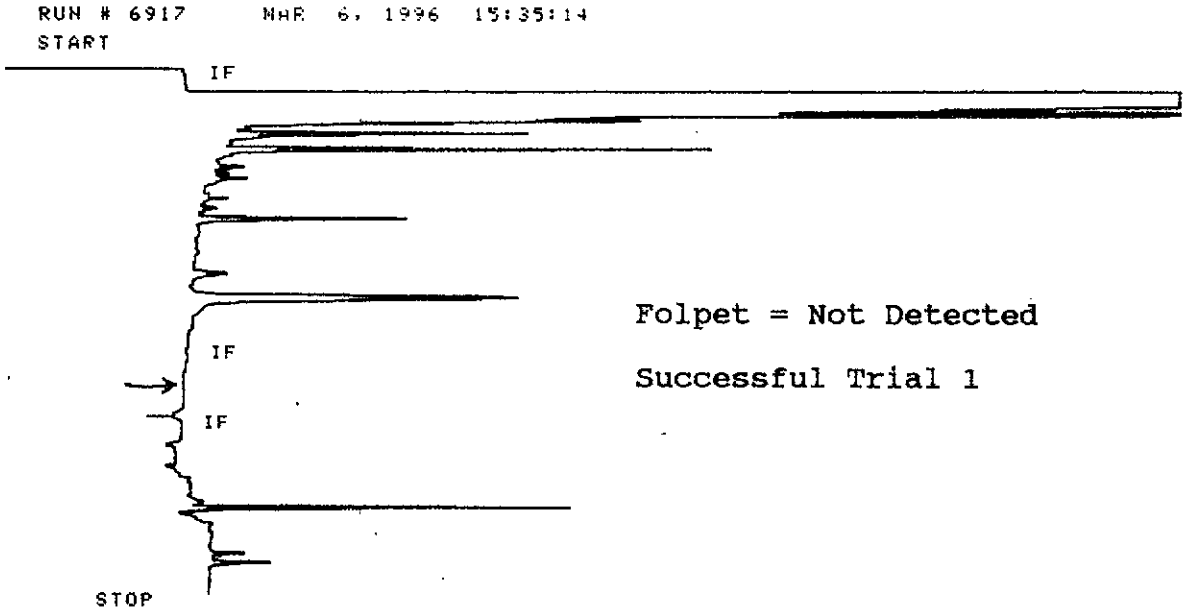
RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
9.378	PB	663217	.152	72676		.000	
9.912	BB	62956	.164	6409	1	.000	FOLPET

TOTAL HEIGHT= 79085
MUL FACTOR=1.0000E+00

①Ⓔ KEN 2-28-96

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #1 - Cranberries
10146-073 Control Cranberries
Inj vol 1 μL 4 mL F.V.



Folpet = Not Detected

Successful Trial 1

RUN# 6917 MAR 6, 1996 15:35:14

SAMPLE# 3

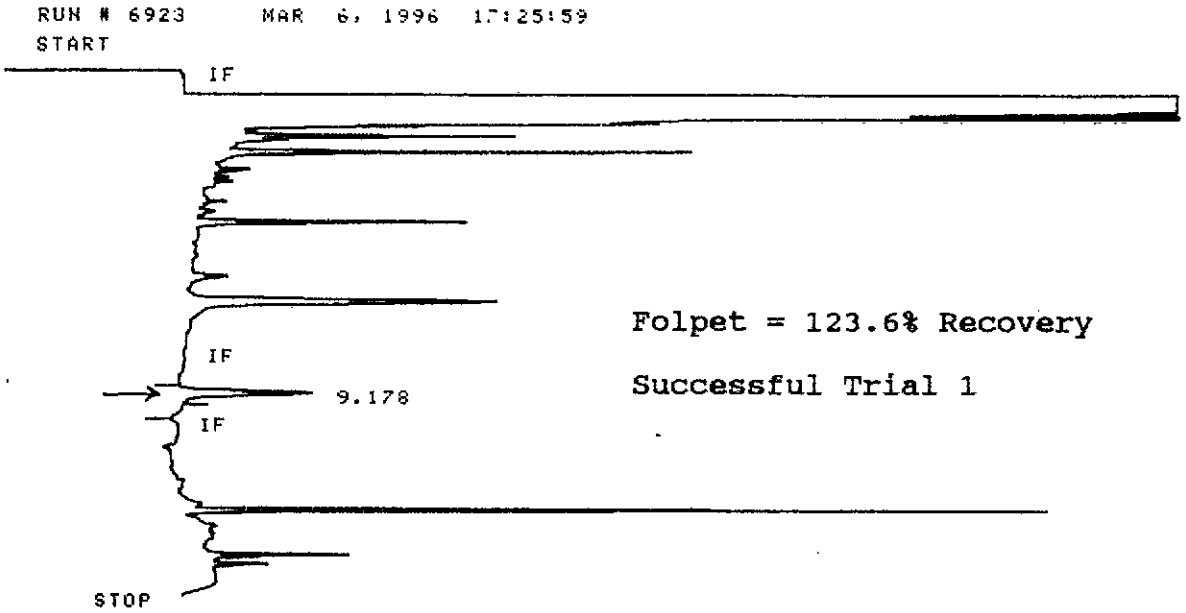
METHOD NAME: M*FOLPET2A.MET

IDENTIFIER : GC#529
NO RUN PEAKS STORED

BEST AVAILABLE COPY

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #1 - Cranberries
10146-075 Control Cranberries + 0.05 ppm
Inj vol 1 μL 4 mL F.V.



RUN# 6923 MAR 6, 1996 17:25:59

SAMPLE# 9

METHOD NAME: M*FOLPET2A.MET

IDENTIFIER : GC#529
ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CHL#	AMOUNT	NAME
9.178	BB	154856	.161	16020	1	.000	FOLPET

TOTAL AREA= 154856
MUL FACTOR=1.0000E+00

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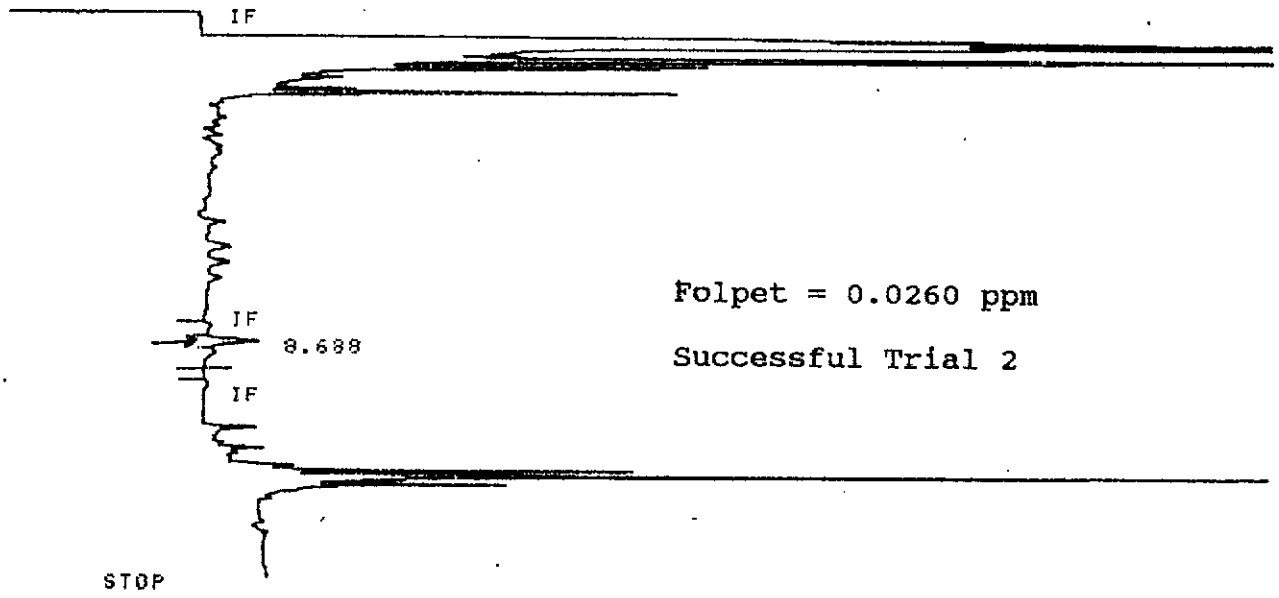
Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #2 - Avocados
10146-089 Control Avocados
Inj vol 1 μL 6 mL F.V.

RUN # 7617 APR 15, 1996 17:50:48
START



RUN# 7617 APR 15, 1996 17:50:48

SAMPLE# 3
METHOD NAME: M*FOLPET2A.MET

IDENTIFIER : GC#529
ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CHL#	AMOUNT	NAME
8.688	VV	60352	.158	6371	1R	.000	FOLPET

TOTAL AREA= 60352
MUL FACTOR=1.0000E+00

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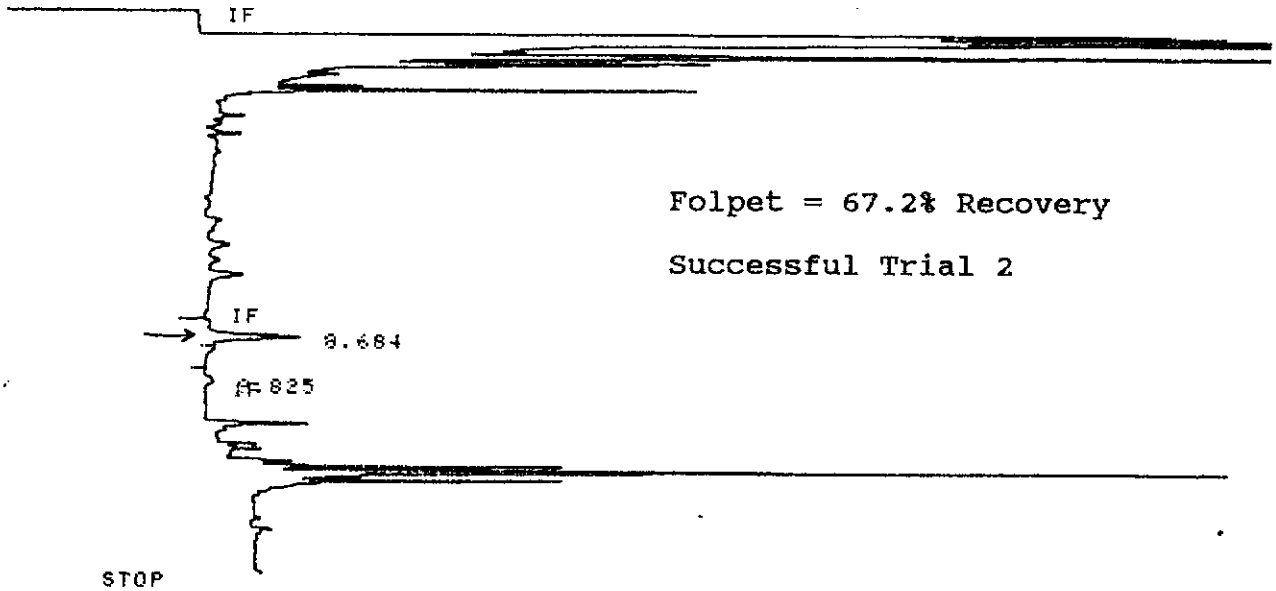
Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number
Method Trial #2 - Avocados
10146-091 Control Avocados + 0.05 ppm
Inj vol 1 μL 6 mL F.V.

RUN # 7623 APR 15, 1996 19:41:29
START



Folpet = 67.2% Recovery

Successful Trial 2

RUN# 7623 APR 15, 1996 19:41:29

SAMPLE# 9

METHOD NAME: M*FOLPET2A.MET

IDENTIFIER : GC#529
ESTD-AREA

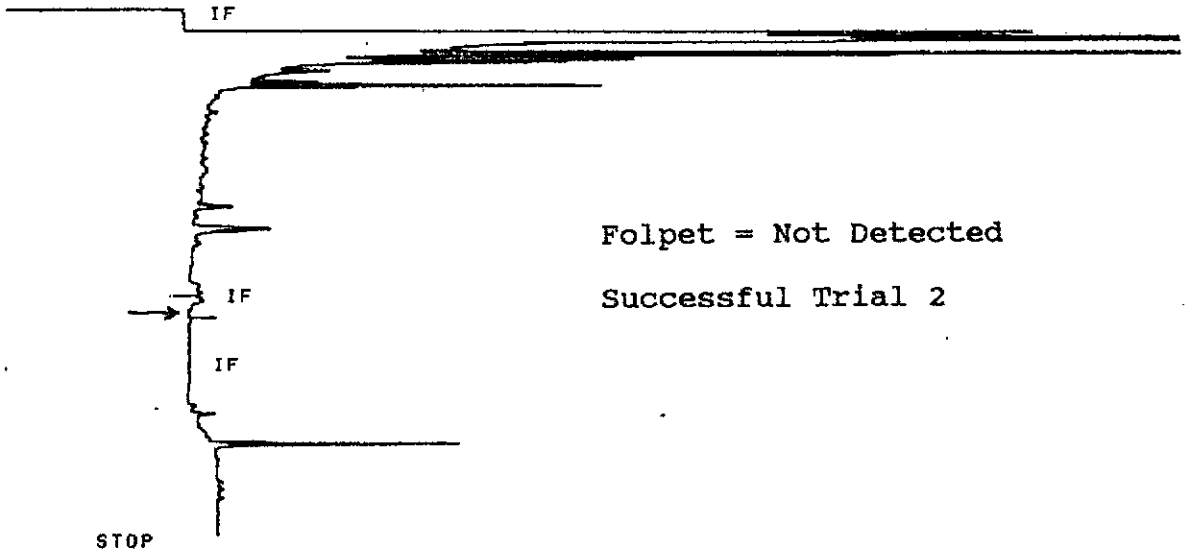
RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
8.684	BV	117108	.182	10736	1R	.000	FOLPET
9.825	I VH	16058	.247	1084		.000	

TOTAL AREA= 133166
MUL FACTOR=1.0000E+00

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #2 - Onions
10146-~~033~~ 037 Control Onions
Inj vol 1 μL 4 mL F.V.

RUN # 7637 APR 15, 1996 23:59:23
START



Folpet = Not Detected

Successful Trial 2

RUN# 7637 APR 15, 1996 23:59:23

SAMPLE# 23

METHOD NAME: M*FOLPET2A.MET

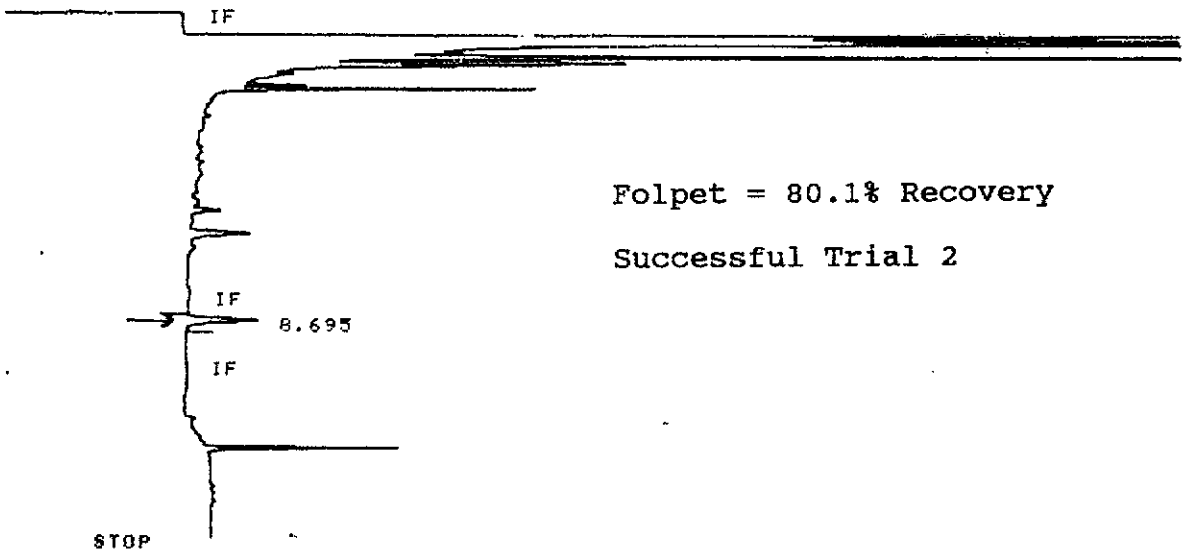
IDENTIFIER : GC#529
NO RUN PEAKS STORED

①ⓔREH 4-16-96

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.

Horizon Protocol Number 10146
Method Trial #2 - Onions
10146-035 Control Onions + 0.05 ppm
Inj vol 1 μL 4 mL F.V.

RUN # 7643 APR 16, 1996 01:49:40
START



Folpet = 80.1% Recovery

Successful Trial 2

RUN# 7643 APR 16, 1996 01:49:40

SAMPLE# 29

METHOD NAME: M*FOLPET2A.MET

IDENTIFIER : GC#529
ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CHL#	AMOUNT	NAME
8.695	PB	76905	.147	8747	1R	.000	FOLPET

TOTAL AREA= 76905
MUL FACTOR=1.0000E+00

(1)(E) REH 4-16-96

BEST AVAILABLE COPY

Source Document: "Independent Laboratory Confirmation of Analytical Methods for the Determination of Folpet in Plant Tissues", by M. Williams, Horizon Laboratories, Inc., Columbia, MO, Study #10146.